

## RESEARCH ARTICLE

# Sec71 separates Golgi stacks in *Drosophila* S2 cells

Syara Fujii<sup>1</sup>, Kazuo Kurokawa<sup>2</sup>, Tatsuya Tago<sup>1</sup>, Ryota Inaba<sup>1</sup>, Arata Takiguchi<sup>1</sup>, Akihiko Nakano<sup>2</sup>, Takunori Satoh<sup>1,\*</sup> and Akiko K. Satoh<sup>1,\*</sup>

## ABSTRACT

Golgi stacks are the basic structural units of the Golgi. Golgi stacks are separated from each other and scattered in the cytoplasm of *Drosophila* cells. Here, we report that the ARF-GEF inhibitor Brefeldin A (BFA) induces the formation of BFA bodies, which are aggregates of Golgi stacks, *trans*-Golgi networks and recycling endosomes. Recycling endosomes are located in the centers of BFA bodies, while Golgi stacks surround them on their *trans* sides. Live imaging of S2 cells revealed that Golgi stacks repeatedly merged and separated on their *trans* sides, and BFA caused successive merger by inhibiting separation, forming BFA bodies. S2 cells carrying genome-edited BFA-resistant mutant Sec71<sup>M717L</sup> did not form BFA bodies at high concentrations of BFA; S2 cells carrying genome-edited BFA-hypersensitive mutant Sec71<sup>F713Y</sup> produced BFA bodies at low concentrations of BFA. These results indicate that Sec71 is the sole BFA target for BFA body formation and controls Golgi stack separation. Finally, we showed that impairment of Sec71 in fly photoreceptors induces BFA body formation, with accumulation of both apical and basolateral cargoes, resulting in inhibition of polarized transport.

**KEY WORDS:** Brefeldin A, Sec71, Golgi ribbon, *Trans*-Golgi network, Recycling endosome, *Drosophila*

## INTRODUCTION

The Golgi is a membrane-bound organelle that is central to the secretory pathway and functions in the modification and sorting of secretory proteins and lipids to multiple destinations within the cell (Klumperman, 2011; Papanikou and Glick, 2014). The basic structural unit of the Golgi is the Golgi stack, composed of multiple flattened cisternae, tubules and vesicles. In plant, *Caenorhabditis elegans*, and *Drosophila* cells, Golgi stacks are separated from each other and scattered in the cytoplasm (Gosavi and Gleeson, 2017; Wei and Seemann, 2017; Yadav and Linstedt, 2011). In contrast, in mammalian cells dozens of Golgi stacks are connected by lateral links to form a Golgi ribbon, which resides in the vicinity of the centrosome via microtubule-based motors. Neither the mechanisms underlying nor the functional significance of ribbon formation have been resolved (Saraste and Prydz, 2019).

*Trans*-Golgi networks (TGNs), located on the *trans* sides of Golgi stacks, were originally considered to be the sorting centers for newly synthesized proteins destined for distinct cellular locations (Kienzle and von Blume, 2014; Luini and Parashuraman, 2016). Recycling endosomes (REs) are perinuclear compartments through which endocytosed materials are trafficked before being recycled back to the plasma membrane (Mayor et al., 1993; Yamashiro and Maxfield, 1987). However, both the TGN and RE are considered hubs of the exocytotic and endocytic pathways (Goldenring, 2015; Hierro et al., 2015; Makaraci and Kim, 2018).

We recently reported that REs are attached to the *trans* sides of Golgi stacks both in *Drosophila* and microtubule-disrupted HeLa cells (Fujii et al., 2020). REs can exist in two distinct, yet interchangeable states, Golgi-associated REs (GA-REs) and free REs – both undergo repeated detachment and reattachment. Moreover, free REs themselves divide and fuse together repeatedly. Since two distinct states of the TGN, Golgi-associated (GA-TGN) and free, are well established in plants (Kang et al., 2011; Uemura et al., 2019, 2014; Viotti et al., 2010), we propose that the plant TGN might be an equivalent organelle to the animal RE (Fujii et al., 2020).

Brefeldin A (BFA) is a fungal toxin that affects exocytotic and endocytic membrane trafficking in eukaryotes by inhibiting guanine-nucleotide exchange factors that regulate ARF GTPases (Anders and Jürgens, 2008; Casanova, 2007; Peyroche et al., 1996; Shin and Nakayama, 2004). In most mammalian cells, except for canine MDCK cells, BFA prevents the binding of peripheral COPI proteins to Golgi membranes and causes intensive tubule extension from Golgi membranes and redistribution of Golgi-resident proteins into the ER (Cole et al., 1996; Donaldson et al., 1990; Lippincott-Schwartz and Liu, 2006; Lippincott-Schwartz et al., 1989; Orci et al., 1991; Sciaky et al., 1997). In addition to its effect on the early secretory pathway, BFA induces the fusion of TGN, endosomes and lysosomes, forming tubule networks emanating from the juxtannuclear region (Hunziker et al., 1991; Lippincott-Schwartz et al., 1991; Wood et al., 1991). This endosomal impact of BFA is observed even in MDCK cells and is partly induced by the inhibition of  $\gamma$ -adaptin [the  $\gamma$  subunit of adapter protein complex 1 (AP1 $\gamma$ )] binding to TGN (Futter et al., 1998; Hunziker et al., 1991; Ishizaki et al., 2008). Tobacco BY2 and yeast cells react to BFA in a manner similar to mammalian cells: Golgi disruption, redistribution of Golgi-resident proteins into the ER, and fusion of TGN/endosomes, which are separated from the early secretory compartments (Hicke et al., 1997; Ito et al., 2017; Langhans et al., 2011; Peyroche et al., 1996; Yasuhara and Shibaoka, 2000; Yasuhara et al., 1995). In *Arabidopsis*, BFA does not induce Golgi absorption into the ER, but instead the Golgi and TGN aggregate, forming ‘BFA compartments’ or ‘BFA bodies’ (Dragwidge et al., 2019; Geldner et al., 2003; Robinson et al., 2008; Uemura and Nakano, 2013). Interestingly, in *Arabidopsis*, TGN markers aggregate at the centers of BFA bodies, whereas Golgi markers localize to the periphery (Robinson et al., 2008; Uemura and Nakano, 2013).

<sup>1</sup>Program of Life and Environmental Science, Graduate School of Integral Science for Life, Hiroshima University, 1-7-1 Kagamiyama, Higashi-Hiroshima, Hiroshima 739-8521, Japan. <sup>2</sup>Live Cell Super-Resolution Imaging Research Team, RIKEN Center for Advanced Photonics, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan.

\*Authors for correspondence (aksatoh@hiroshima-u.ac.jp; tsatoh3@hiroshima-u.ac.jp)

© K.K., 0000-0003-3549-4795; T.T., 0000-0002-9745-8063; A.T., 0000-0001-5257-0644; A.N., 0000-0003-3635-548X; T.S., 0000-0003-0340-5532; A.K.S., 0000-0001-7336-6642

In *Drosophila*, BFA inhibits cellularization (Sisson et al., 2000) and plasma membrane transport of Delta (Kondylis and Rabouille, 2003); Golgi stacks are not disrupted, rather they form clusters or aggregates (Kondylis and Rabouille, 2009; Kondylis et al., 2007; Xu et al., 2002). However, *cis-trans* polarity in Golgi aggregation and the effects of BFA on the TGN or REs have not been well investigated. Here, we report that TGN/REs repeatedly merge and separate, and that BFA inhibits TGN/RE separation by inactivating the function of Sec71, a *Drosophila* guanine-nucleotide exchange factor (GEF) for ARF GTPases, resulting in the formation of BFA bodies. TGN/RE aggregates are located at the centers of BFA bodies and Golgi stacks surround the aggregate on their *trans* sides. The Golgi/RE organization of BFA bodies in S2 cells resembles that of normal COS-1 cells, suggesting that the morphological and structural organization of Golgi/RE reflects the kinetics of TGN/RE merger and separation in cells.

## RESULTS

### BFA-induced aggregation of Golgi stacks maintains *cis-trans* polarity in S2 cells

To investigate the effects of BFA on Golgi stacks and the TGN/REs, we incubated S2 cells expressing the medial-Golgi marker mannosidase II::GFP (ManII::GFP) or monomeric Turquoise2::Rab11 (mTq2::Rab11) for 2 h with or without 50  $\mu$ M BFA at 25°C, followed by immunostaining with anti-GM130 (a *cis*-Golgi marker) and anti-Rab6 (TGN marker) antibodies (Fig. 1A,B). Approximately 30% of BFA-treated S2 cells showed aggregation of Golgi stacks, TGN and REs. Both mTq2::Rab11 and Rab6 localized to the central domain of the aggregate, but there was a clear difference in the localization of mTq2::Rab11, which was observed only at the central core, whereas Rab6 localized more broadly. In untreated cells, Rab11 localized specifically to the RE, whereas Rab6 was broadly distributed from *trans*-Golgi to the RE. The arrangement of ManII, Rab6, and Rab11 in BFA-induced aggregates likely reflects that in normal cells. GM130 localized only to the periphery of the aggregate, while ManII::EGFP was located between GM130 and the peripheral edge of Rab6 (Fig. 1A). The *trans*-Golgi markers ST::EGFP and GalT::EGFP colocalized with Rab6, but displayed stronger signals in the periphery than in the central region (Fig. 1C,D). The TGN marker Golgin245 colocalized very well with Rab6, but more weakly in the most central part of the aggregate (Fig. 1D). These results indicate that *cis-trans* polarity of Golgi stacks is generally maintained in BFA-induced aggregates, and that the *trans* sides of Golgi stacks are facing the central core formed by TGN/REs (Fig. 1E). Therefore, we describe BFA-induced aggregates in S2 cells as BFA bodies since their organization resembles that described in plant cells, in which TGN forms the center and *trans*-Golgi markers are located in the periphery (Robinson et al., 2008; Uemura et al., 2014).

Additionally, we investigated the membrane structure of BFA bodies by electron microscopy (EM) and a genetic EM-tag, APEX2 (Martell et al., 2017). Previously, we showed that DAB deposition generated by GalT::APEX2::EGFP is limited to the lumen of *trans*-Golgi cisternae in S2 cells (Fujii et al., 2020). In S2 cells in the absence of BFA, Golgi stacks with DAB deposition in *trans* cisternae were scattered in the cytoplasm; however, in S2 cells incubated for 2 h with BFA, Golgi stacks were not clear, but cisternae and tubules with DAB deposition were gathered in one place (Fig. 1F,G), where cisternae and tubule-networks likely represent BFA bodies and resemble those in plants (Geldner et al., 2003; Robinson et al., 2008).

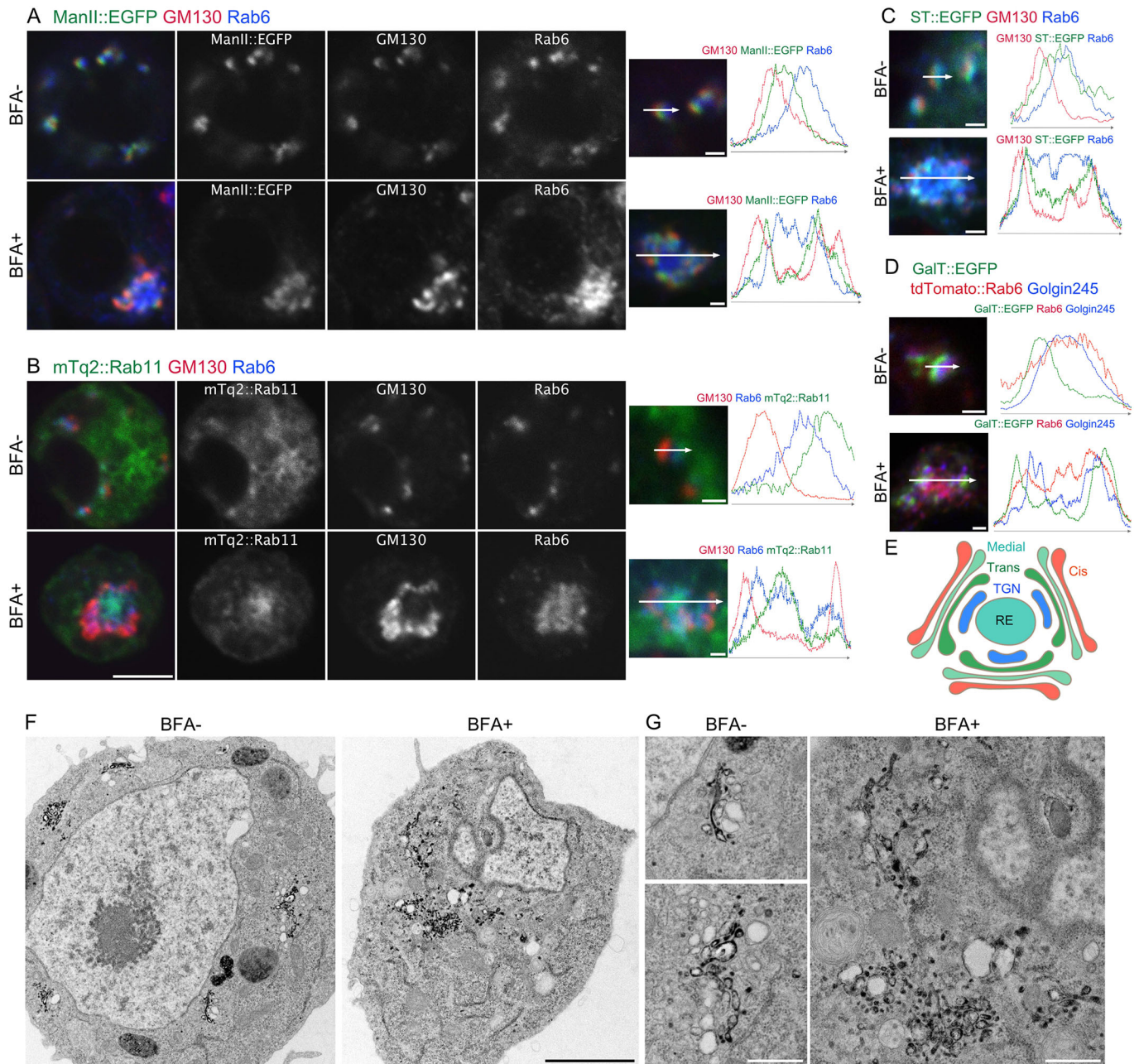
As Golgi stacks in mammalian cells are gathered near centrosomes via microtubules, we determined whether microtubules are necessary

for BFA body formation in S2 cells. Most *Drosophila* cells, including S2 cells, have no functional centrosomes during interphase (Rogers et al., 2008; Rusan and Rogers, 2009); however, S2 cells assemble functional centrosomes during mitosis. Thus, we first compared the positions of BFA bodies and microtubule asters spreading from functional centrosomes at early prophase, and found that BFA bodies were not located near microtubule asters (Fig. S2A, upper panels). We also closely investigated the positioning of the BFA body and microtubule lattice at interphase (Fig. S2A, panels in middle row). Although some tdTomato::Rab6-positive tubules extended along with microtubules outside the BFA body (Fig. S2A, panels in middle row, arrow), the BFA body itself was not tightly associated with microtubules. We further examined the effect of the microtubule-polymerization inhibitor colchicine on BFA body formation, and found that BFA bodies could be formed in the absence of microtubules (Fig. S2A, lower panels). Interestingly, there were small fragmented microtubules near Golgi stacks and other places following colchicine treatment that might correspond to previously reported microtubule nucleation sites on Golgi stacks or unidentified sites throughout the cytoplasm (Rogers et al., 2008; Rusan and Rogers, 2009). These results indicate that microtubules are not necessary for BFA body formation.

### Impairment of Sec71 function induces BFA body formation in S2 cells

From numerous studies on yeast, human, and plant cells, it is well known that BFA targets ARF-GEFs belonging to the Sec7/BIG and Gea/GBF families (Jackson, 2018; Peyroche et al., 1996; Sata et al., 1998; Wright et al., 2014). In the *Drosophila* genome, there is only one member of the Sec7/BIG family ARF-GEFs, namely, Sec71 (Cox et al., 2004), and a single member of the Gea/GBF family, ARF-GEF, namely Garz (Cox et al., 2004; Wang et al., 2012). Thus, we investigated whether impairment of Sec71 or Garz function caused BFA body formation. First, we examined the localization of overexpressed wild-type V5::Sec71 and V5::Garz, as well as their effects on Golgi organization. As reported previously (Armbruster and Luschnig, 2012; Christis and Munro, 2012; Wang et al., 2012, 2017), V5::Sec71 localized at the *trans* sides, while V5::Garz localized at the *cis* sides of Golgi stacks, with neither influencing their organization (Fig. 2A,B, upper panels, 2D,E, and Fig. S2C,D, upper panels, Fig. S2E,G). Next, we investigated the effects of V5::Sec71<sup>E677K</sup> or V5::Garz<sup>E740K</sup> expression. These mutants have been reported to impair GEF activity and function in a dominant-negative manner (Armbruster and Luschnig, 2012; Wang et al., 2017). The expression of V5::Sec71<sup>E677K</sup> induced Golgi stacks to aggregate (Fig. 2A, lower panels), with Rab6 localized broadly to the interior and GM130 peripherally (Fig. 2F). GalT::mTq2 signals were dominant in peripheral foci, but also were found in the interiors of aggregates (Fig. S2C, lower panels, Fig. S2F). These distributions were similar to those observed in BFA bodies in S2 cells (Fig. 1A–C). In these aggregates, V5::Sec71<sup>E677K</sup> was located at the center (Fig. 2A, lower panels, Fig. 2F and Fig. S2C, lower panels, Fig. S2F). In contrast, the expression of V5::Garz<sup>E740K</sup> caused the diffusion of Rab6 and GalT::mTq2, although GM130 foci were still visible and colocalized with V5::Garz<sup>E740K</sup> foci, as discussed below (Fig. 2B, Fig. S2D, lower panels). Similar to what is seen upon V5::Sec71<sup>E677K</sup> expression, double-stranded RNA knockdown of Sec71 resulted in Golgi aggregation with a radial polarity similar to that of BFA bodies – a *cis*-Golgi marker GM130 and a medial-Golgi marker p120 (also known as Glg1) (Yamamoto-Hino et al., 2012) localized at the periphery, with TGN markers Rab6 and Golgin245 located at the central region (Fig. 2C, panels in middle row, Fig. 2G–I and Fig. S2B





**Fig. 1. BFA induces the formation of BFA bodies.** (A,B) Left, representative immunostaining of S2 cells expressing ManII::EGFP (A) or mTq2::Rab11 (B) (green) incubated without (upper panels) or with 50  $\mu$ M BFA (lower panels) with anti-GM130 (red) and anti-Rab6 (blue) antibodies. Right panels, plots of signal intensities from image to the left. Signal intensities were measured along the 1.5  $\mu$ m (upper) or 5  $\mu$ m (lower) arrows shown in inset. Graphs show the overlap between channels. (C,D) Left pictures are immunostaining of S2 cells expressing ST::EGFP (green) (C) or GalT::EGFP (green) and tdTomato::Rab6 (red) (D) incubated without (upper panels) or with 50  $\mu$ M BFA (lower panels) by anti-GM130 (red) and anti-Rab6 (blue) antibodies (C) or by anti-Golgin245 antibody (blue) (D). Right plots of signal intensities from image on the left. Signal intensity was measured along the 1.5  $\mu$ m (upper) or 5  $\mu$ m (lower) arrow in inset, graph shows the overlap between channels. (E) Schematic of the structure of a BFA body. (F,G) Electron micrographs of S2 cells expressing GalT::APEX2::EGFP without or with 50  $\mu$ M BFA. GalT::APEX2::EGFP was visualized by osmium-enhanced DAB-depositions. Scale bars: 5  $\mu$ m (A,B, left panels), 1  $\mu$ m (A,B, right panels, C,D), 2  $\mu$ m (F), and 500 nm (G).

panels in middle row). The RE marker mTq2::Rab11 also localized to the central region (Fig. 2I). In contrast, double-stranded RNA knockdown of Garz induced the diffusion of p120, Rab6 and Golgin245, but not of GM130 (Fig. 2C, lower panels; Fig. S2B, lower panels). These results confirmed that these phenotypes were caused by the impairment of Sec71 or Garz.

We investigated GalT::APEX2::EGFP-positive membrane structures in S2 cells expressing V5::Sec71<sup>E677K</sup> or V5::Garz<sup>E740K</sup>

by electron microscopy. In V5::Sec71<sup>E677K</sup>-expressing S2 cells, the cisternae and tubules with DAB deposition gathered in one place (Fig. 2J). This phenotype resembles BFA-treated S2 cells (Fig. 1F,G), whereas in V5::Garz<sup>E740K</sup>-expressing S2 cells, ER and vesicles with DAB deposition were amplified, with no cytologically recognizable Golgi stacks (Fig. 2K). These results indicate that Sec71 loss-of-function phenocopies BFA treatment and results in structures similar to BFA bodies.



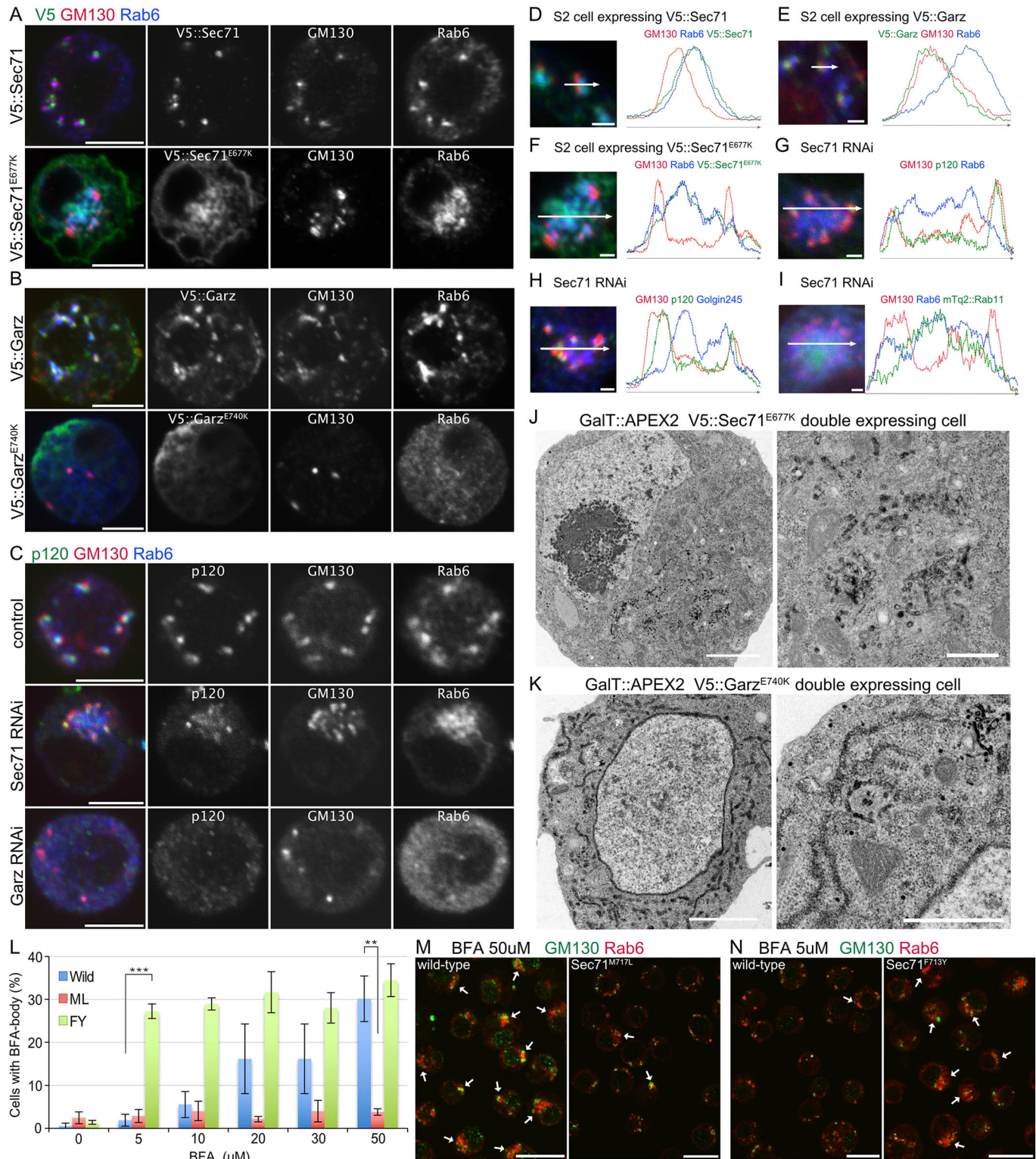


Fig. 2. See next page for legend.

### Sec71 is the sole BFA target in BFA body formation in S2 cells

BFA binds to the Sec7 domain of ARF-GEFs. Biochemical experiments, crystal structure analysis, and sequence alignment of BFA-sensitive and -insensitive ARF-GEFs revealed that the critical amino-acid residues in the Sec7 domain responsible for BFA binding; Y190, S191, M194, T197 and V204 in the Sec7 domain inhibit GDP/GTP exchange activity of ARF-GEF

(Geldner et al., 2003; Peyroche et al., 1999; Renault et al., 2003; Viaud et al., 2007; Zeeh et al., 2006). We edited the genome sequence of Sec71 by CRISPR/Cas9-mediated knock-in, and generated two types of S2 cells with Sec71 point mutations, Sec71<sup>M717L</sup>, which lacks the conserved M194 in the Sec7 domain and is expected to be BFA resistant, and Sec71<sup>F713Y</sup>, which gains



### Fig. 2. Sec71 is the only BFA target in BFA body formation in S2 cells.

(A) Immunostaining of cells expressing V5::Sec71 (upper panels) or V5::Sec71<sup>E677K</sup> (lower panels) by anti-V5 (green), anti-GM130 (red), and anti-Rab6 (blue) antibodies. (B) Immunostaining of cells expressing V5::Garz (upper panels) or V5::Garz<sup>E740K</sup> (lower panels) by anti-V5 (green), anti-GM130 (red), and anti-Rab6 (blue) antibodies. (C) Immunostaining of cells transfected with double-stranded RNA against Sec71 (panels in middle row) or Garz (lower panels) or not transfected (upper panels) by anti-p120 (green), anti-GM130 (red), and anti-Rab6 (blue) antibodies. (D–I) Left, immunostaining of cells expressing V5::Sec71 (D), V5::Garz (E), V5::Sec71<sup>E677K</sup> (F), mTq2::Rab11 (green) (I), or transfected with double-stranded RNA against Sec71 (G–I). Anti-V5 (green), anti-GM130 (red), and anti-Rab6 (blue) antibodies (D–F). Anti-p120 (green), anti-GM130 (red), and anti-Rab6 (blue) antibodies (G). Anti-p120 (green), anti-GM130 (red), and anti-Golgin245 (blue) antibodies (H). Anti-GM130 (red) and anti-Rab6 (blue) antibodies (I). Right, plots of signal intensities from images on the left. Signal intensities were measured along the 1.5  $\mu\text{m}$  (D,E) and 5  $\mu\text{m}$  (F–I) arrows shown in insets. Graphs show the overlap between channels. (J,K) Electron micrographs of cells co-expressing GalT::APEX2::EGFP and V5::Sec71<sup>E677K</sup> (J) or V5::Garz<sup>E740K</sup> (K). GalT::APEX2::EGFP was visualized by osmium-enhanced DAB-deposition. (L) Percentages of wild-type, Sec71<sup>M717L</sup> and Sec71<sup>F713Y</sup> S2 cells with BFA bodies after 2 h of incubation with 0, 5, 10, 20, 30 and 50  $\mu\text{M}$  BFA. BFA bodies were defined as focused Rab6 staining surrounded by GM130 staining. Results are mean $\pm$ s.d. for  $n > 250$  in three independent experiments. (M) Immunostaining of wild-type cells (left) and genome-edited cells expressing BFA-resistant mutant Sec71<sup>M717L</sup> (right) treated with 50  $\mu\text{M}$  BFA by anti-GM130 (green) and anti-Rab6 (red) antibodies. (N) Immunostaining of wild-type cells (left) and genome-edited cells with BFA-hypersensitive mutant Sec71<sup>F713Y</sup> (right) treated with 5  $\mu\text{M}$  BFA with anti-GM130 (green) and anti-Rab6 (red) antibodies. Scale bars: 5  $\mu\text{m}$  (A–C), 1  $\mu\text{m}$  (D–I), 2  $\mu\text{m}$  (J,K, left), 500 nm (J,K, right), 20  $\mu\text{m}$  (M,N). \*\* $P < 0.01$ , \*\*\* $P < 0.001$  (unpaired two-tailed Student's *t*-test).

the consensus sequence of Y190 on the Sec7 domain, which is expected to be more sensitive to BFA than the wild-type protein (Peyroche et al., 1999), and is therefore referred to as hypersensitive.

We investigated the sensitivity to BFA of these two mutant and wild-type S2 cells (Fig. 2L–N). S2 cells with Sec71<sup>M717L</sup> did not respond to BFA even at a high concentration (50  $\mu\text{M}$ ) – BFA bodies were formed in 3.8 $\pm$ 0.73% of Sec71<sup>M717L</sup> S2 cells and 30.2 $\pm$ 5.3% of wild-type S2 cells (mean $\pm$ s.d.; Fig. 2L,M). In contrast, S2 cells with Sec71<sup>F713Y</sup> responded to BFA at a low concentration (5  $\mu\text{M}$ ) – BFA bodies were formed in 27.2 $\pm$ 1.7% of Sec71<sup>F713Y</sup> S2 cells and 1.9 $\pm$ 1.4% of wild-type S2 cells (Fig. 2L,N). Thus, substitutions of one amino acid in Sec71 alone can dramatically change the BFA sensitivity of S2 cells. Notably, the proportion of Sec71<sup>F713Y</sup> S2 cells with BFA bodies did not dramatically increase with increasing BFA doses from 5  $\mu\text{M}$  (27.2 $\pm$ 1.7%) to 50  $\mu\text{M}$  (34.4 $\pm$ 3.8%) (Fig. 2L). Moreover, wild-type S2 cells with BFA bodies reached a similar proportion at 50  $\mu\text{M}$  (30.2 $\pm$ 5.3%) (Fig. 2L). These results indicate that the effect of BFA is likely to reach a maximum at 5  $\mu\text{M}$  for cells with Sec71<sup>F713Y</sup> mutant and 50  $\mu\text{M}$  for the wild-type Sec71. These results indicate that impairment of Sec71 is necessary and sufficient for BFA body formation. Thus, Sec71 is the only BFA target responsible for BFA body formation.

### Sec71 localized to the center of BFA bodies

We examined the localization of endogenous Sec71 using an anti-Sec71 antibody (Wang et al., 2017). As previously reported (Christis and Munro, 2012; Wang et al., 2017), wild-type Sec71 colocalized with the TGN markers Golgin245 and tdTomato::Rab6, and also localized between the *cis*-Golgi marker GM130 and the RE marker Rab11, indicating that endogenous Sec71 is on the TGN (Fig. 3A,C,E, upper panels and plots). In wild-type BFA bodies, Sec71 was extensively concentrated at centers (Fig. 3A,C,E, lower

panels and plots). Rab11 and tdTomato::Rab6 also localized to the centers of BFA bodies but more broadly than Sec71 (Fig. 3C,E, lower panels and plot). Golgin245 was mostly found between GM130 and Sec71 (Fig. 3A, lower panels and plots). As shown above, the polarity of Golgi stacks is largely maintained in BFA bodies, as *cis* outwards and *trans* inwards (Fig. 1). These results indicate that BFA uncouples Sec71 from two other TGN markers, Rab6 and Golgin245, and induces Sec71 aggregation, resulting in BFA body formation.

To investigate whether Sec71<sup>F713Y</sup> and wild-type Sec71 behave in a similar manner, except for their sensitivities to BFA, we compared the distributions of Sec71 and Golgi/RE markers in both untreated and BFA-treated Sec71<sup>F713Y</sup> cells (Fig. 3B,D,F,H–J) with those in untreated and BFA-treated wild-type cells (Fig. 3A,C,E,G; Fig. 1C,D). The distributions of GM130, Golgin245, Rab11, GalT::EGFP, tdTomato::Rab6, tdTomato::Rab11, ST::EGFP and Sec71 in Sec71<sup>F713Y</sup> cells were similar to those in wild-type control cells. The sizes and shapes of Sec71<sup>F713Y</sup> BFA bodies were not obviously different from those of wild-type BFA bodies.

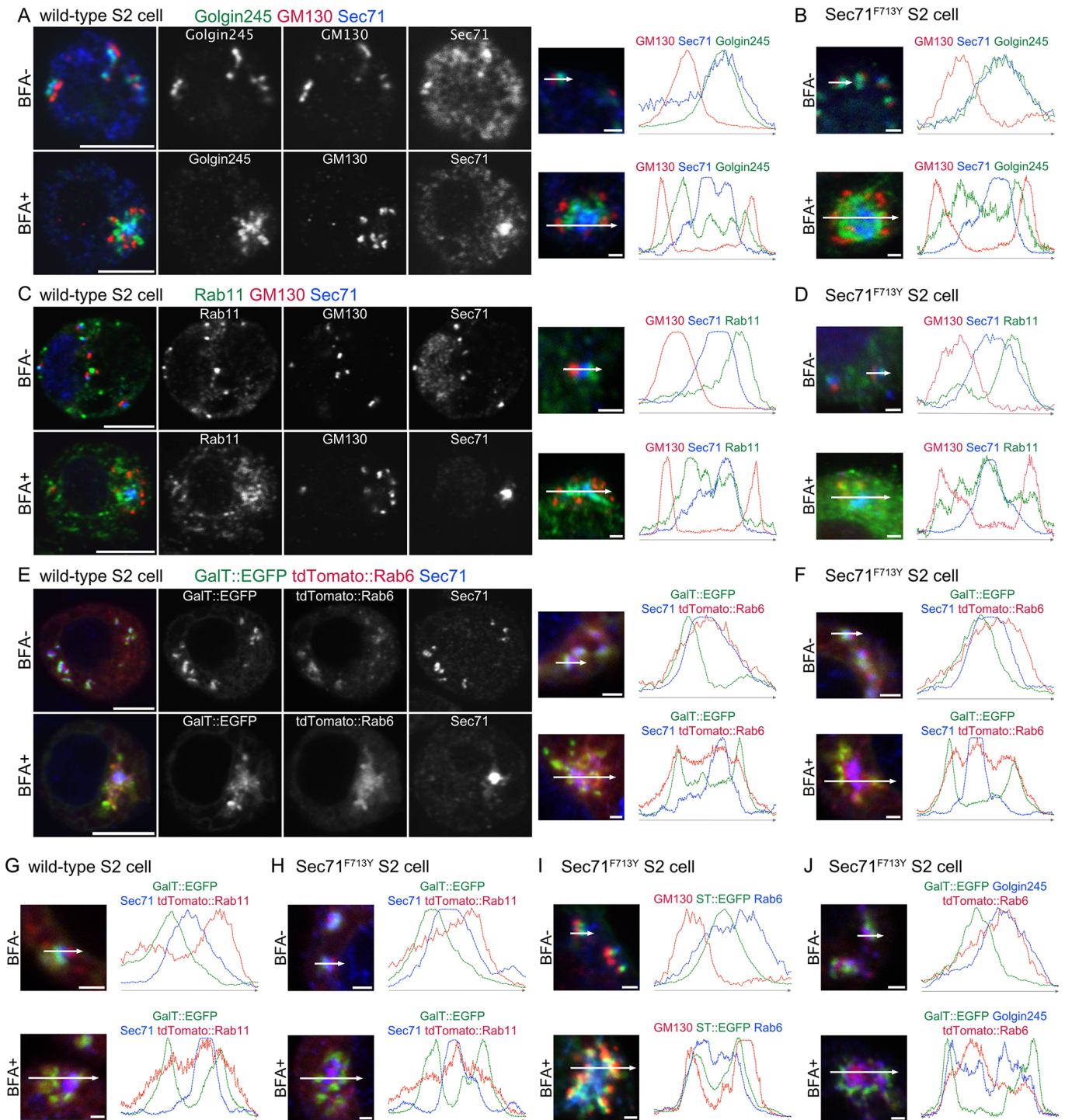
### Live imaging of BFA body formation in S2 cells

To understand the mechanism of BFA body formation, we used confocal microscopy. To avoid using high BFA concentrations, we used BFA-hypersensitive Sec71<sup>F713Y</sup> S2 cells coexpressing *trans*-Golgi (GalT::EGFP) and TGN markers (tdTomato::Rab6) (Fig. 4A,B; Movies 1–3). In BFA-untreated cells, Golgi stacks move slowly but constantly, sometimes hitting each other and occasionally connecting by the merger of their TGNs (Fig. 4A, arrows; Movie 1). Separation of these TGN-connected Golgi stacks was also often observed (Fig. 4A, arrowheads; Movie 1). The mergers and separations of TGNs were confirmed in the *XY* and *XZ* projections of 3D time-lapse observations (Movies 2 and 3).

The merger and separation of TGNs was constantly repeated in BFA-untreated cells – 4.86 $\pm$ 1.81 TGN merger and 4.57 $\pm$ 1.72 TGN separation events were observed in five optical sections at a 0.5- $\mu\text{m}$  interval of a cell within 60 min (mean $\pm$ s.d.; Fig. 4C). After BFA addition, TGN mergers were similar to those of BFA untreated cells, but TGN separation was limited; 5.43 $\pm$ 0.90 TGN mergers and 1.29 $\pm$ 0.59 TGN separations occurred in the same conditions (Fig. 4C). The ratio of TGN mergers to TGN separations per cell increased from 1.09 $\pm$ 0.28 to 4.23 $\pm$ 1.34 upon BFA addition (Fig. 4C). As a result, Golgi stacks developed aggregates connected via TGNs at their cores (Fig. 4B; Movies 1 and 3). These observations indicate that BFA inhibits the separation of TGN, leading to the formation of BFA bodies.

To understand how BFA affects Sec71 dynamics, Sec71 movement before and after BFA addition was examined by super-resolution confocal live imaging microscopy (SCLIM). Before BFA treatment, iRFP::Sec71 fluorescence was relatively strong on the *trans* sides of GalT::EGFP-positive *trans*-Golgi membranes; however, the shapes of the iRFP-positive structures were unstable and most of the iRFP signal appeared hazy in the cytoplasm (Fig. 4E; Movie 4).

Minutes after the addition of BFA, as the dispersed iRFP signals decreased, iRFP::Sec71 became concentrated to form stable globular structures, locating near to GalT::EGFP-positive *trans*-Golgi membranes but not in direct contact. Both *trans*-Golgi and Sec71-positive structures were accompanied by tdTomato::Rab6, which often filled the space between them (Fig. 4F; Movie 4). At 24 and 79 min (Fig. 4G,H) after BFA addition, cells had aggregates of iRFP::Sec71 that were surrounded by GalT::EGFP-positive *trans*-Golgi membranes connected via tdTomato::Rab6 (Fig. 4G,H;



**Fig. 3. Sec71 is localized to the center of the BFA body.** Immunostaining and plots of signal intensities from wild-type (A,C,E,G) and Sec71<sup>F713Y</sup> S2 cells (B,D,F,H–J). Right, plots of signal intensities from images on the left. Signal intensities were measured along the 1.5  $\mu$ m (upper panels) and 5  $\mu$ m (lower panels) arrows shown in insets. In all panels, cells not treated with BFA are shown in the upper row and BFA-treated cells are shown in the lower row. 50  $\mu$ M and 25  $\mu$ M BFA was used for the wild-type cells and Sec71<sup>F713Y</sup> S2 cells, respectively. (A,B) Immunostaining with anti-Golgin245 (green), anti-GM130 (red), and anti-Sec71 (blue) antibodies. (C,D) Immunostaining with anti-Rab11 (green), anti-GM130 (red), and anti-Sec71 (blue) antibodies. (E,F) S2 cells expressing GalT::EGFP (green) and tdTomato::Rab6 (red) immunostained with anti-Sec71 (blue) antibody. (G,H) S2 cells expressing GalT::EGFP (green) and tdTomato::Rab11 (red) immunostained with anti-Sec71 (blue) antibody. (I) S2 cells expressing ST::EGFP (green) immunostained with anti-GM130 (red) and anti-Rab6 (blue) antibodies. (J) S2 cells expressing GalT::EGFP (green) and tdTomato::Rab6 (red) immunostained with anti-Golgin245 (blue) antibody. Scale bars: 5  $\mu$ m (A,C,E, left panels), 1  $\mu$ m (A,C,E, right panels, B,D,F,G–J).

Movie 4). These results indicate that BFA inhibits the dynamic behavior of Sec71, which results in the aggregation of Sec71-positive structures and Golgi stacks. This is consistent with the

mechanism of action of BFA, which inhibits the turnover of ARF GTPases by stabilizing the complex of GDP-ARF–ARF-GEF, in the case of *Drosophila*, Sec71 on the TGN.



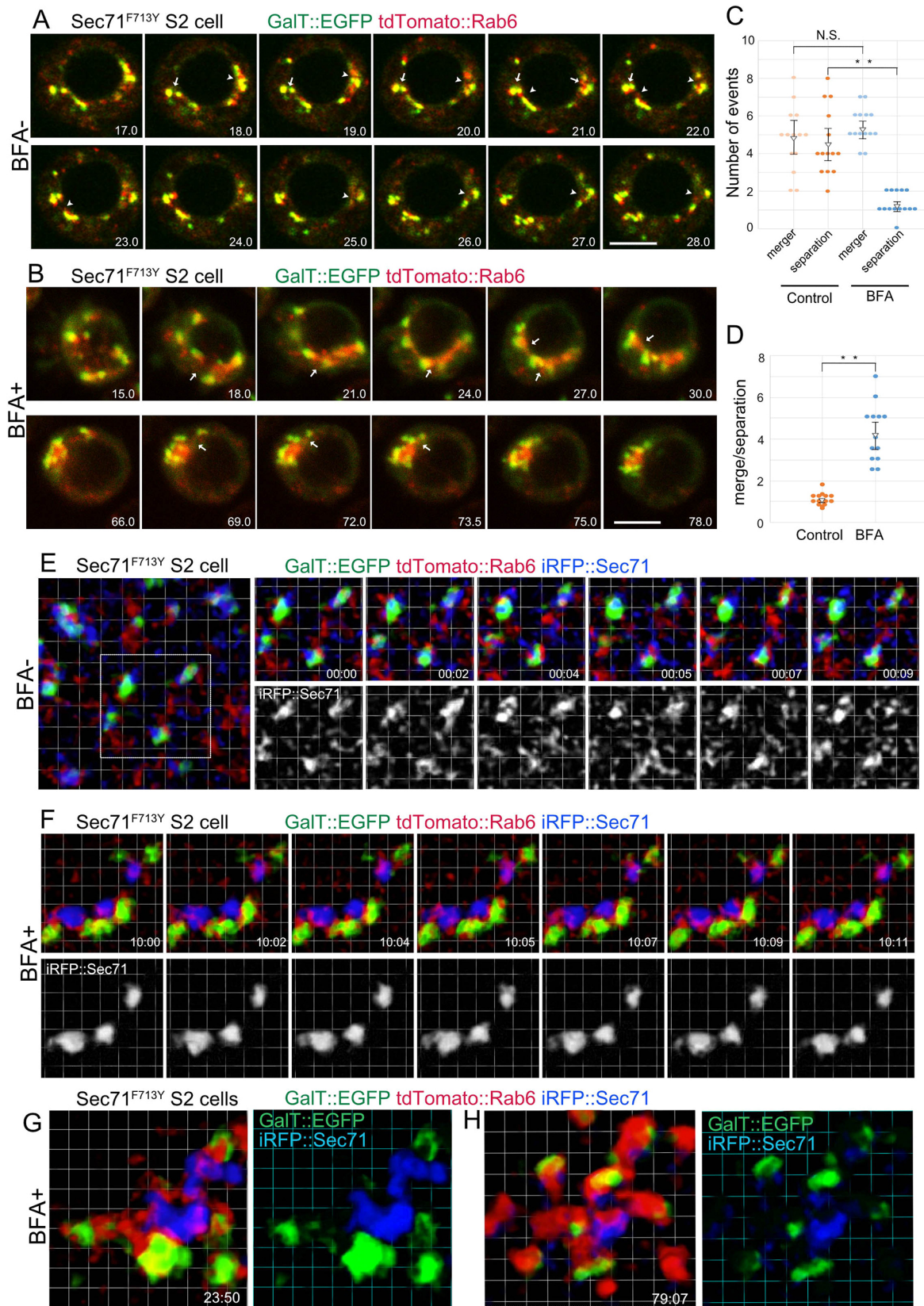


Fig. 4. See next page for legend.

### Sec71 exclusively localizes to TGN in fly photoreceptors

To understand the function of Sec71 in fly photoreceptors, we first examined the localization of endogenous Sec71. In contrast to

Rab11, which is located not only on the *trans* sides of Golgi stacks, but also on post-Golgi vesicles at the base of the rhabdomeres (Otsuka et al., 2019; Satoh et al., 2005), Sec71 associated

**Fig. 4. Live imaging of BFA body formation.** (A,B) Frames from time-lapse movies of BFA-hypersensitive S2 cells carrying the Sec71<sup>F713Y</sup> substitution, and also expressing GalT::EGFP (green) and tdTomato::Rab6 (red) with (B) or without 25  $\mu$ M BFA treatment (A) observed by confocal microscopy. Numbers at right bottom corners indicate the time (min) after BFA addition (B) or start point of time-lapse observation (A). Arrows or arrowheads indicate mergers or separations of TGN of two or more Golgi stacks. (C) Quantifications of mergers or separations of TGNs in untreated (left) and BFA-treated S2 cells (right). (D) Ratios of mergers to separations of TGNs in untreated (left) and BFA-treated (right) S2 cells carrying the Sec71<sup>F713Y</sup> substitution. Error bars show mean $\pm$ s.d. (E–H) Frames of time-lapse movies of S2 cells carrying the BFA-hypersensitive Sec71<sup>F713Y</sup> substitution, also expressing GalT::EGFP (green), tdTomato::Rab6 (red) and iRFP::Sec71 (blue) with (F–H) or without 25  $\mu$ M BFA treatment (E) observed by SCLIM. Numbers at the right bottom corners indicate the time (min:sec) after BFA addition (F–H) or start point of time-lapse observation (E). Scale bars: 5  $\mu$ m (A,B); Grid: 1  $\mu$ m (E–H). \*\* $P$ <0.01, N.S., not significant (unpaired two-tailed Student's  $t$ -test).

exclusively with the Golgi stack/GA-RE complex (Fig. 5A,B). Detailed analysis indicated that Sec71 localization within the Golgi stack/GA-RE complex was on the TGN (Fig. 5C–G), that is, the *trans* side of the *cis*-Golgi marker Rab1 (Fig. 5G), the medial-Golgi markers p120 and metallophosphoesterase (MPPE) (Fig. 5C,E,F) (Chen et al., 2005) and the *trans*-Golgi marker GalT::ECFP (Fig. 5D), and *cis*-side of the RE marker, Rab11 (Fig. 5E). Sec71 strongly colocalized with the TGN marker Golgin245 (Fig. 5F), but more to the *cis* side of another TGN marker, AP1 $\gamma$  (Fig. 5G) (Hirst et al., 2009). These results suggest that Sec71 mainly functions on the TGN of photoreceptors.

#### Rh1 and Na<sup>+</sup>K<sup>+</sup>-ATPase accumulated in Golgi aggregates in Sec71-deficient photoreceptors

We next investigated the effects of Sec71 impairment in fly photoreceptors. We first expressed dominant-negative Sec71 (Sec71<sup>E677K</sup>) by means of an Rh1-Gal4 driver, which induces the expression in R1–6 peripheral photoreceptors from late pupal stages. In Sec71<sup>E677K</sup>-expressing late pupal photoreceptors, the TGN marker Rab6 and the medial-Golgi marker MPPE colocalized on the entire Golgi aggregate (Fig. 6A). Most of Rab11, an RE marker, seemed to be diffused, but some staining remained in the Golgi aggregate (Fig. 6B). The *cis*-Golgi markers GM130 and Syntaxin5::myc (Syx5::myc) (Norgate et al., 2010) localized to the peripheries of aggregates (Fig. 6C,D). As ARF-GEFs recruit coat proteins to Golgi stacks, we investigated the localization of a subunit of COPI,  $\alpha$ COP (Kitazawa et al., 2012) and AP1 $\gamma$  (Hirst et al., 2009). Both  $\alpha$ COP and AP1 $\gamma$  localized on Golgi stacks in wild-type photoreceptors (Fig. 6E,F, upper panels). However, AP1 $\gamma$  was completely diffuse in Sec71<sup>E677K</sup>-expressing R1–6 peripheral photoreceptors, although  $\alpha$ COP localized peripherally in Golgi aggregates, similar to other *cis*-Golgi markers (Fig. 6E,F, lower panels). Notably, punctate staining of AP1 $\gamma$  was found in R7 cells, which do not express Sec71<sup>E677K</sup>. On the other hand, in Garz<sup>E740K</sup> expressing photoreceptors, Rab6, MPPE,  $\alpha$ COP, and AP1 $\gamma$  all were diffuse (Fig. S3A–D). The diffuse Rab6 and MPPE colocalized with ER markers Calnexin99A (Cnx) (Rosenbaum et al., 2006) and ER-membrane complex subunit 3 (EMC3) (Sato et al., 2015), respectively (Fig. S3E,F). Garz<sup>E740K</sup> likely induced redistribution of Golgi resident proteins into the ER. Interestingly, GM130 staining still remained punctate; we discussed this issue below. Thus, the phenotypes of photoreceptors expressing Sec71<sup>E677K</sup> and Garz<sup>E740K</sup> correspond to those of S2 cells under Sec71 and Garz impairment, respectively.

Recently, RNAi-based screening identified Sec71 as an important factor for apical trafficking in fly photoreceptors

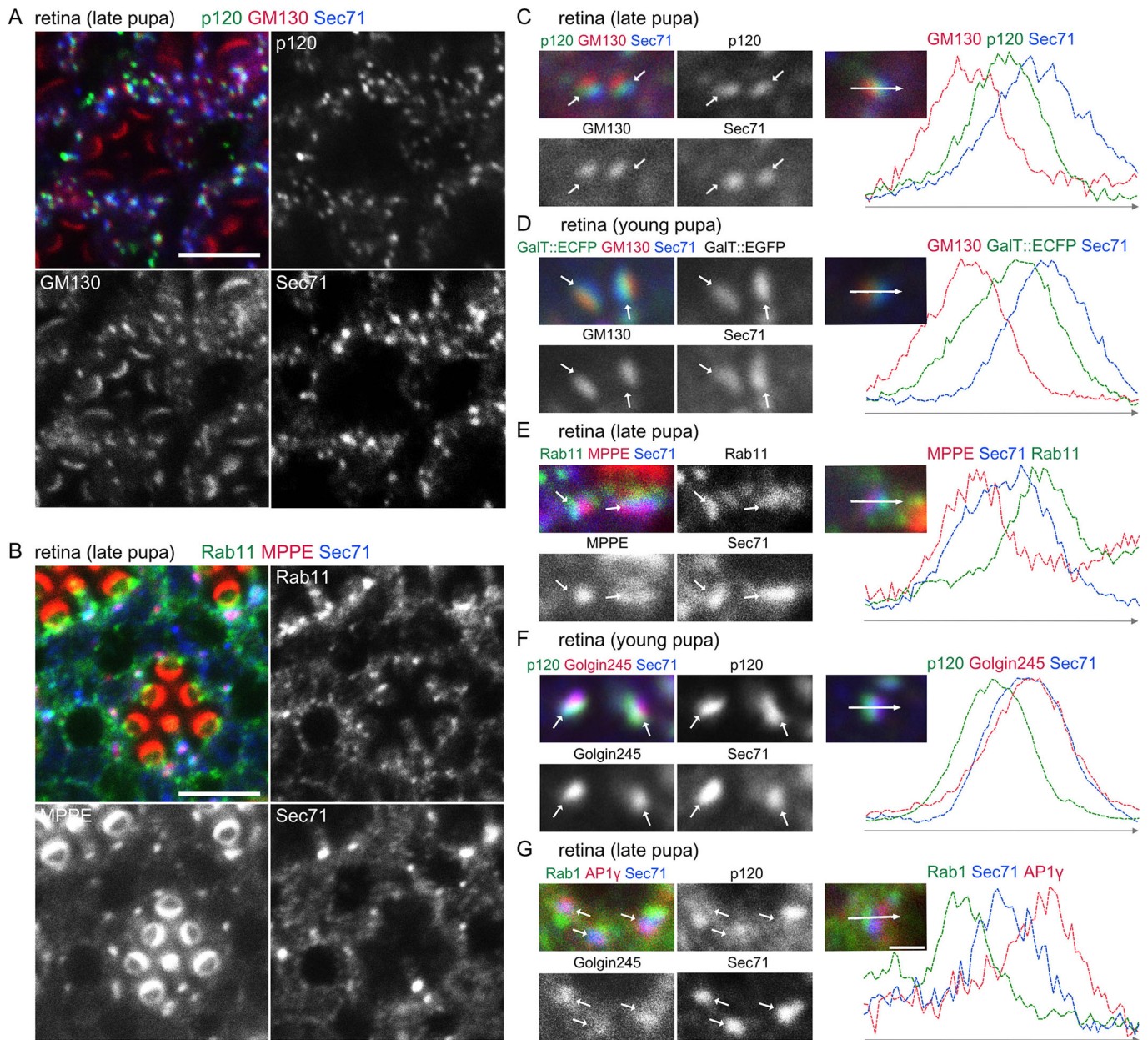
(Laffafian and Tepass, 2019). Moreover, Sec71 was shown to be essential not only for apical transport, including Rh1 and Eys, but also for basolateral transport of Na<sup>+</sup>K<sup>+</sup>-ATPase. Similarly, we found that Rh1 accumulation in the rhabdomere was severely inhibited by Sec71<sup>E677K</sup> expression induced by Rh1-Gal4 (Fig. 6G). However, the defect in Na<sup>+</sup>K<sup>+</sup>-ATPase transport was limited in these photoreceptors (Fig. 6G), as Rh1-Gal4 expression probably occurs too late to sufficiently inhibit Na<sup>+</sup>K<sup>+</sup>-ATPase transport. Interestingly, we found accumulation of robust Rh1 and some Na<sup>+</sup>K<sup>+</sup>-ATPase in Golgi aggregates (Fig. 6G,H). The staining of Rh1 and Na<sup>+</sup>K<sup>+</sup>-ATPase partially overlapped but clearly separated within Golgi aggregates, suggesting that sorting of these proteins might be done without Sec71 (Fig. 6H).

To verify the function of Sec71 in fly photoreceptors, we attempted to create mosaic eyes containing Sec71-deficient clones using a strong mutant allele, *Sec71<sup>ex11</sup>*, using the FRT-FLP system. However, we failed to obtain *Sec71<sup>ex11</sup>* homozygous clones, suggesting the lethality of Sec71 deficiency. Thus, we knocked down Sec71 using RNAi. To make mosaic retinas, we used coin-FLP-Gal4 (Bosch et al., 2015) with *eyeless*-FLP, and expressed Sec71-RNAi<sup>GLC01657</sup> or Sec71-RNAi<sup>HMS00357</sup> from early eye development. These two RNAi lines target different parts of the *Sec71* mRNA (see Flybase). Sec71-RNAi<sup>GLC01657</sup>-expressing photoreceptors had Rab6-positive aggregates surrounded by GM130-positive foci (Fig. 6I). The transport of Rh1 and Na<sup>+</sup>K<sup>+</sup>-ATPase was severely inhibited (Fig. 6J). Sec71-RNAi<sup>HMS00357</sup>-expressing photoreceptors also showed severe Rh1 and Na<sup>+</sup>K<sup>+</sup>-ATPase defects (Fig. 6K). These phenotypes of photoreceptors expressing Sec71-RNAi are consistent with those of cells expressing Sec71<sup>E677K</sup>. Unlike ommatidia expressing Sec71<sup>E677K</sup>, the inter-rhabdomeral space (IRS) of Sec71-RNAi-expressing ommatidia was not fully open (Fig. 6I–K). As the IRS is formed by the secretion of Eys from the apical membrane of photoreceptors (Husain et al., 2006; Laffafian and Tepass, 2019), we investigated the localization of Eys (Fig. 6L). It accumulated in the cytoplasm of the Sec71-RNAi<sup>GLC01657</sup>-expressing photoreceptors, with IRSs that were small or difficult to recognize. This difference in phenotypes between cells expressing Sec71<sup>E677K</sup> and cells expressing Sec71-RNAi could be explained by differences in the onset of Sec71 deficiency, such that Rh1Gal4-driven Sec71<sup>E677K</sup> inhibits Sec71 function starting from the late pupal stage, whereas coin-FLP-Gal4-driven Sec71-RNAi knockdown starts from early in eye development. Eys occasionally accumulated in the cytoplasm with Rh1 in cells expressing Sec71<sup>E677K</sup> (Fig. 6M), supporting this hypothesis. These results indicate that Sec71-deficiency inhibits anterograde transport to the apical and basolateral membrane as well as the secretion to the IRS.

#### Tubule network and vesicle accumulation in Sec71-deficient photoreceptors

To investigate membrane structure in Sec71-deficient photoreceptors, we observed thin sections of Rh1-Gal4/+, Rh1-Gal4/UAS-Sec71<sup>E677K</sup>, and coin-FLP-Gal4/UAS-Sec71-RNAi<sup>GLC01657</sup> pupal photoreceptors using electron microscopy (Fig. 7). In wild-type photoreceptors, the rhabdomeres were round-shaped (Fig. 7A,D), but in Sec71-deficient photoreceptors they were not round, as well as being rather smaller and thinner than in wild-type cells (Fig. 7B,C,E,F). Several ER membrane sheets and Golgi stacks were observed in typical thin sections of wild-type photoreceptors (Fig. 7D, arrows, arrowhead). However, in Sec71<sup>E677K</sup>-expressing photoreceptors, the ER membrane was amplified and Golgi stacks were not observed; instead, tubule networks and vesicles were





**Fig. 5. Sec71 exclusively localizes to TGN in photoreceptors.** Immunostaining of retinas dissected from wild-type young and late-pupal flies with (A) anti-p120 (green), anti-GM130 (red) and anti-Sec71 (blue) antibodies; (B) anti-Rab11 (green), a medial-Golgi marker, anti-MPPE (red), and anti-Sec71 (blue) antibodies. Anti-MPPE antibody stains not only the medial Golgi but also the tips of the rhabdomeres. It is not known whether the latter staining represents genuine MPPE localization. (C–G) Left, immunostaining of retinas by the indicated antibodies. GalT::ECFP was expressed in D. Right, plots of signal intensities from images to the left. Signal intensities were measured along the 1.5  $\mu$ m arrow in the insets; graphs show the overlap between channels. Scale bars: 5  $\mu$ m (A,B), 1  $\mu$ m (C–G).

often observed (Fig. 7E, arrow, 7H,J,K). The amplification of ER membrane and tubule networks was also observed in Sec71-RNAi photoreceptors (Fig. 7F, arrows, 7I,L). The accumulation of cytoplasmic Rh1 and Na<sup>+</sup>K<sup>+</sup>-ATPase likely localizes on these tubule networks and vesicles, which are most probably Golgi aggregates. Consistent with our confocal microscopy observations, the IRS of Sec71-RNAi ommatidia was found to be quite small and fragmented (Fig. 7C,F).

These results indicate that structures of ER and Golgi stacks are strongly affected, and transport for both apical and basolateral membrane domains is inhibited in Sec71-deficient photoreceptors.

## DISCUSSION

### Impairment of Sec71 function results in TGN/RE aggregation, leading to formation of BFA bodies

We showed that the ARF-GEF inhibitor BFA induces the formation of BFA bodies in *Drosophila* cells (Fig. 1). Detailed analysis of BFA bodies using confocal microscopy indicates that aggregated TGN/RE is central and Golgi stacks are located at the periphery of BFA bodies (Fig. 1). We also showed that the sensitivity of S2 cells to BFA could be completely controlled by genome editing of a single ARF-GEF gene, Sec71; S2 cells carrying the BFA-resistant Sec71<sup>M717L</sup> mutation do not form BFA bodies even at a high BFA concentration, whereas S2 cells carrying BFA-hypersensitive



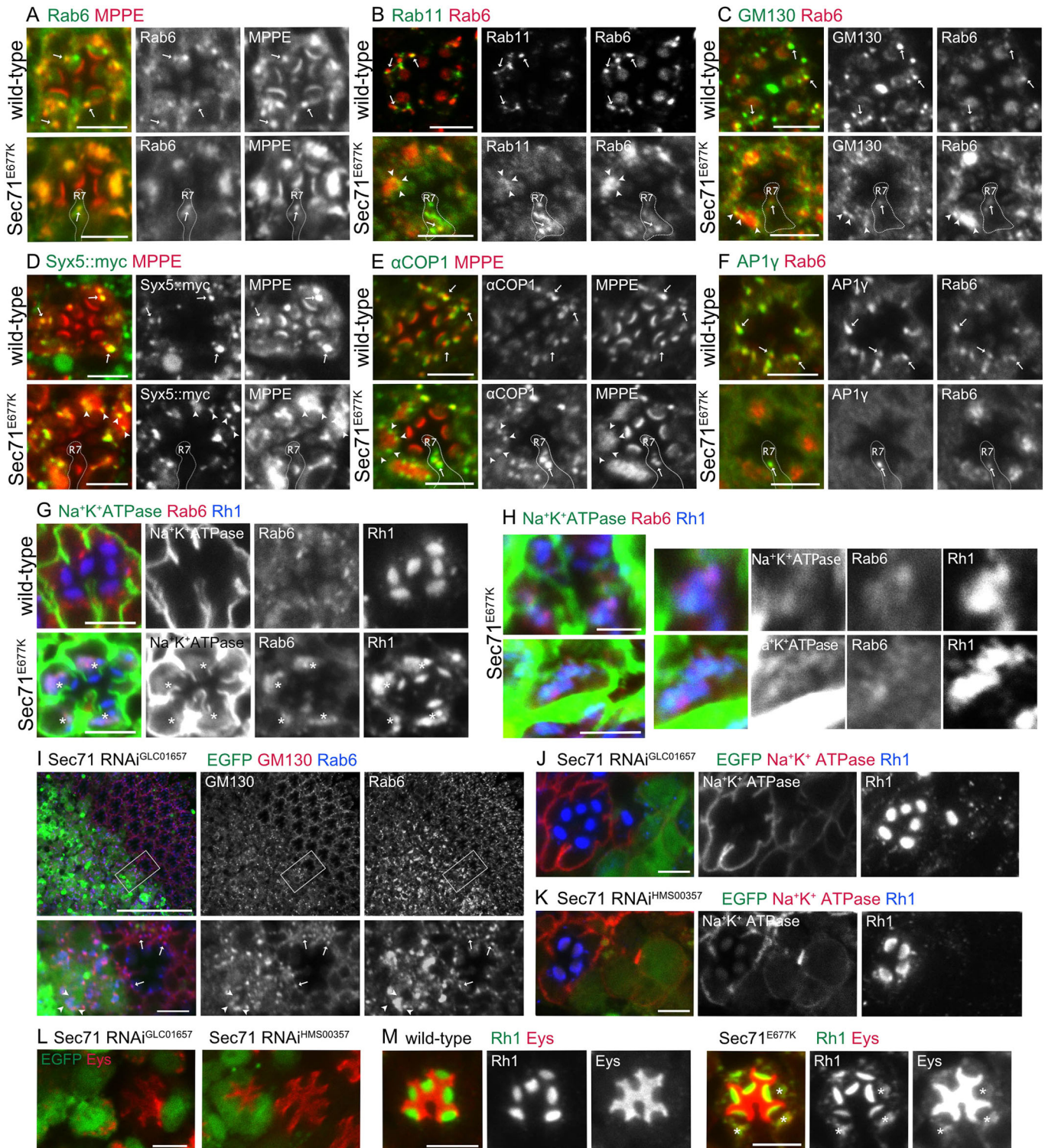


Fig. 6. See next page for legend.

Sec71<sup>F713Y</sup> form BFA bodies at low BFA concentration (Fig. 2L–N). These results indicate that Sec71 is the sole BFA target for BFA body formation in S2 cells. In addition, BFA-hypersensitive S2 cells provide an excellent model system to study the role of ARF-GEF and ARF1 in the TGN.

In normal S2 cells, Golgi stacks are moving constantly and often fuse together on their TGNs, and then separate spontaneously (Fig. 4A,C; Movies 1–3). We previously reported that REs are

attached to the *trans* sides of Golgi stacks in both *Drosophila* and microtubule-disrupted HeLa cells (Fujii et al., 2020). Moreover, REs and Golgi stacks as well as free REs themselves undergo repeated detachment and reattachment. Although RE detachment and reattachment are much more rapid events than the TGN mergers and separations of Golgi stacks reported here, RE dynamics could be the basis of Golgi stack attachment to their TGNs. In BFA-treated S2 cells, TGN/RE separations were suppressed but TGN/RE-



**Fig. 6. Basolateral and rhabdomere transport is inhibited in Sec71<sup>E677K</sup>-expressing photoreceptors.** (A–G) Immunostaining of retinas from Rh1-Gal4/+ (upper panels) and Rh1-Gal4/UAS- Sec71<sup>E677K</sup> (lower panels) late-pupal flies. Sec71<sup>E677K</sup> is expressed in the R1–6 peripheral photoreceptors (A–C, E–G). Immunostaining of retinas from Rh1-Gal4/UAS-Syx5::myc (upper panels) and Rh1-Gal4/UAS-Syx5::myc, UAS- Sec71<sup>E677K</sup> (lower panels) late-pupal flies. Sec71<sup>E677K</sup> is expressed in the R1–6 peripheral photoreceptors (D). Anti-Rab6 (green) and anti-MPPE (red) antibodies (A). Anti-Rab11 (green) and anti-Rab6 (red) antibodies (B). Anti-GM130 (green) and anti-Rab6 (red) antibodies (C). Anti-myc (green) and anti-MPPE (red) antibodies (D). Anti- $\alpha$ COPI (green) and anti-MPPE (red) antibodies (E). Anti-AP1 $\gamma$  (green) and anti-Rab6 (red) antibodies (F). Anti-Na<sup>+</sup>K<sup>+</sup>-ATPase- $\alpha$  (green), anti-Rab6 (red), and anti-Rh1 (blue) antibodies (G). Arrows indicate Golgi stacks in the wild-type cells, and arrowheads indicate the foci of *cis*-Golgi makers surrounding the medial and late Golgi aggregates. Cytoplasmic accumulation of Rh1 is indicated by asterisks. (H) Immunostaining of retinas from Rh1-Gal4/UAS-Sec71<sup>E677K</sup> late-pupal flies using anti-Na<sup>+</sup>K<sup>+</sup>-ATPase- $\alpha$  (green), anti-Rab6 (red), and anti-Rh1 (blue) antibodies. (I–L) Immunostaining of retinas dissected from Sec71-RNAi<sup>GLC01657</sup> (I, J, L, left) or Sec71RNAi<sup>HMS00357</sup> (K, L, right) mosaic retinas. GFP marks Sec71-RNAi-expressing cells. Anti-GM130 (red) and anti-Rab6 (blue) antibodies (I). Anti-Na<sup>+</sup>K<sup>+</sup>-ATPase- $\alpha$  (red) and anti-Rh1 (blue) antibodies (J, K). Anti-Eys antibody (red) (L). Arrows indicate Golgi stacks in the wild-type cells, and arrowheads indicate the foci of *cis*-Golgi makers surrounding Golgi aggregates. (M) Immunostaining of retinas from Rh1-Gal4/+ (left) and Rh1-Gal4/UAS- Sec71<sup>E677K</sup> (right) late-pupal flies. Sec71<sup>E677K</sup> is expressed in the R1–6 peripheral photoreceptors. Anti-Rh1 (green) and anti-Eys (red) antibodies. Cytoplasmic accumulation of Rh1 and Eys is indicated by asterisks. Scale bars: 5  $\mu$ m (A–G), 2  $\mu$ m (H), 50  $\mu$ m (I, upper), 5  $\mu$ m (I, lower, J–M).

mergers were not affected, resulting in the formation of BFA bodies (Fig. 4B–D). Around the *trans* sides of Golgi stacks in normal S2 cells, Sec71 appears to be dynamic and unstable (Fig. 4E). BFA quickly stabilizes Sec71 on the TGN/RE and Sec71 finally localizes to the center of BFA bodies (Fig. 4F–H). Sec71 has been shown to accelerate the transport carrier formation at the TGN/RE through ARF1 activation (Christis and Munro, 2012; Wang et al., 2017). Together with the function of Sec71 already indicated or expected from the studies of homologs (Casanova, 2007; Ishizaki et al., 2008; McDonold and Fromme, 2014; Shin and Nakayama, 2004), we suggest that the deficiency of the transport carrier formation inhibits the separation of TGN/RE, resulting in BFA body formation. The simplified model in Fig. 8 shows that separations and mergers of TGN/RE lead to separation and aggregation of Golgi stacks, respectively, and BFA inhibits Sec71-mediated separations, resulting in BFA body formation.

The appearance of BFA bodies in *Drosophila* S2 cells resembles that of Golgi stacks and REs in many COS-1 cells and some populations of HeLa and MDCK cells, with REs closely associated with the centrosome as one large aggregate and Golgi stacks surrounding the REs with their *trans* sides inward (Misaki et al., 2010). This suggests that one of the determinants of the cell-wide arrangement of Golgi stacks and RE is the kinetic balance between the merger and separation of TGN and REs.

### The sole fly GBF1 ortholog, Garz, is insensitive to BFA

The most prominent effects of BFA on yeast, mammalian cells and tobacco BY2 cells are the breakup of the Golgi and redistribution of Golgi-resident proteins into the ER (Ito et al., 2012; Lippincott-Schwartz et al., 1989; Peyroche et al., 1996; Yasuhara and Shibaoka, 2000; Yasuhara et al., 1995). However, in S2, *Arabidopsis* and maize cells, Golgi-resident proteins were resistant to BFA (Fig. 1) (Baluska et al., 2002; Langhans et al., 2011; Uemura et al., 2014). The effects seen in many cell types can be explained by differences in the BFA sensitivity of GBF1

orthologs (Casanova, 2007; Peyroche et al., 2001, 1996; Robinson et al., 2008; Teh and Moore, 2007; Wright et al., 2014). For example, MDCK cells, in which the Golgi is not absorbed into the ER (Hunziker et al., 1991), have a substitution of one consensus amino acid residue of GBF1 responsible for BFA sensitivity in the Sec7 domain compared to other mammalian GBF1s, resulting in resistance to BFA (Verheije et al., 2008). In this report, we showed that redistribution of Golgi-resident proteins into the ER is not induced by BFA (Fig. 1) but induced by the impairment of Garz activity, as demonstrated by dominant-negative Garz<sup>E740K</sup> expression or knockdown by RNAi in S2 cells (Fig. 2B,C,K; Fig. S2B,D). As Garz lacks the consensus residues required for BFA binding (S191, T197 and V204 in the Sec7 domain), it must be insensitive to BFA. Thus, BFA does not induce breakup of the Golgi in S2 cells.

Neither the expression of dominant-negative Garz<sup>E740K</sup> nor RNAi knockdown in S2 cells or *Drosophila* photoreceptors redistributes GM130, a *cis*-Golgi localizing Golgin, to the ER. The remaining GM130 foci are reminiscent of a previously described structure, the Golgi-entry core compartment (GECCO), small foci containing a particular subset of *cis*-Golgi proteins formed in BFA-treated Tobacco BY2 cells (Ito et al., 2018, 2012). The GECCO was shown to be formed by COPII-independent anterograde transport from the ER, even in the presence of aggressive retrograde traffic induced by GBF1 deficiency. GM130 foci in BFA-treated mammalian cells have also been reported previously (Jiang et al., 2006; Walenta et al., 2001).

### Sec71 is required for polarized transports towards both apical and basolateral domains

Sec71 is the only *Drosophila* ortholog of mammalian BIG1 and BIG2 (also known as ARFGEF1 and ARFGEF2, respectively), which are involved in post-Golgi vesicle formation by recruiting AP1 (Futter et al., 1998). As expected, impairment of Sec71 results in the diffusion of AP1 $\gamma$  in fly photoreceptors (Fig. 6F). We also showed that Golgi stacks and REs form Golgi aggregates, and that *cis*-Golgi markers localize in peripheral regions, similar to BFA bodies (Fig. 6A–F). Consistent with a previous report (Laffafian and Tepass, 2019), the lack of Sec71 inhibits the polarized transport of Rh1 and Na<sup>+</sup>K<sup>+</sup>-ATPase to the apical and basolateral domains (Fig. 6G,H,I,J,K,M). Interestingly, both Rh1 and Na<sup>+</sup>K<sup>+</sup>-ATPase accumulated in Golgi aggregates; however, the two membrane cargoes were segregated from each other, suggesting that sorting might occur even in cells without Sec71 activity (Fig. 6H). We previously showed that GPI, but not VSV-G, localizes to GA-RE, suggesting that segregation of these cargoes occurs at the interface between Golgi stacks and GA-RE (Fujii et al., 2020). Segregation might not require Sec71-mediated coat protein assembly. It is important to determine the exact timing of sorting and carrier formation in future studies.

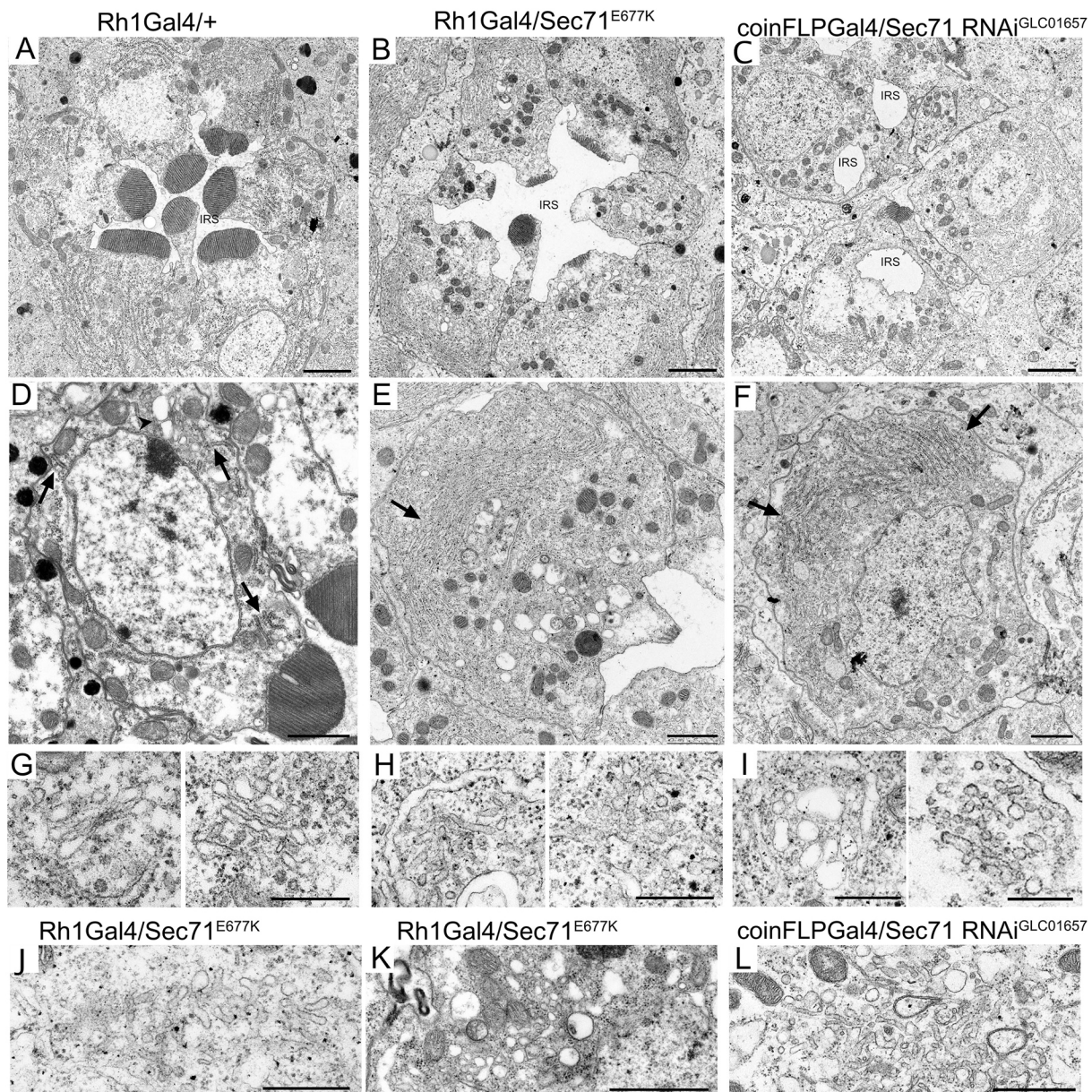
## MATERIALS AND METHODS

### Construction of plasmids

For PCR amplification, the high-fidelity DNA-polymerase KOD plus Neo (TOYOBO, Osaka, Japan) was used, unless otherwise noted. Primers and other oligonucleotides are listed in Table S1. Sequences of plasmids used in this study are shown in Table S2.

The pMK-V5::Sec71 and pMK-V5::Garz plasmids were constructed from cDNAs of total RNA from w<sup>1118</sup> third-instar larvae, produced by PCR using KOD FX Neo (TOYOBO, Osaka, Japan) with primers Sec71-GF1/Sec71-GR2 and Garz-GF1/Garz-GR1, respectively. DNA sequences of 18–20 bp homologous to the vector were added by 10 additional cycles of PCR with primers GL3-Sec71/Sec71-MK-Sp and GL3-garz/garz-MK-Sp, respectively.





**Fig. 7. Tubule networks develop in Sec71-deficient photoreceptors.** Electron micrographs of photoreceptors from Rh1-Gal4/+ (A,D,G), Rh1-Gal4/UAS-Sec71<sup>E677K</sup> (B,E,H,J,K) and coinFLPGal4/UAS-Sec71RNAi<sup>GLC01657</sup> (C,F,I,L) late-pupal flies. (A–C) Electron micrographs of a single ommatidium. The IRS is small and fragmented in C. (D–F) Electron micrographs of single photoreceptors. Arrows show the ER membrane and the arrowhead shows a Golgi stack. (G–I) Electron micrographs of Golgi stacks or related organelles. (J–L) Electron micrographs of tubules and vesicles observed in Sec71-deficient cells. Scale bars: 2 μm (A–C), 1 μm (D–F), 500 nm (G–L).

The DNA fragment encoding the V5 epitope was amplified from pMK-V5::pcs (Otsuka et al., 2019) with primers B-MK-V5 and GL3-R. Using NEBuilder HiFi DNA Assembly Master Mix (NEB, Ipswich, MA, USA), these fragments were assembled between the BamHI and SpeI sites of pMK33-CFH-BD to obtain pMK-V5::Sec71 and pMK-V5::garz. The clones were sequenced using the primers listed below, and one of each clone, without any mutations, was selected and used for subsequent experiments.

pMK-V5::Sec71<sup>E677K</sup> and pMK-V5::garz<sup>E740K</sup> were generated from pMK-V5::Sec71 and pMK-V5::garz using Gibson assembly-mediated site-directed mutagenesis. These V5-tagged genes were amplified with primers K-MT-V5 and MK-MT-Ap and transferred to KpnI-ApaI sites of pMT-puro to construct pMT-hyg-V5::Sec71, pMT-hyg-V5::Sec71<sup>E677K</sup>, pMT-hyg-V5::garz and pMT-hyg-V5::garz<sup>E740K</sup>.

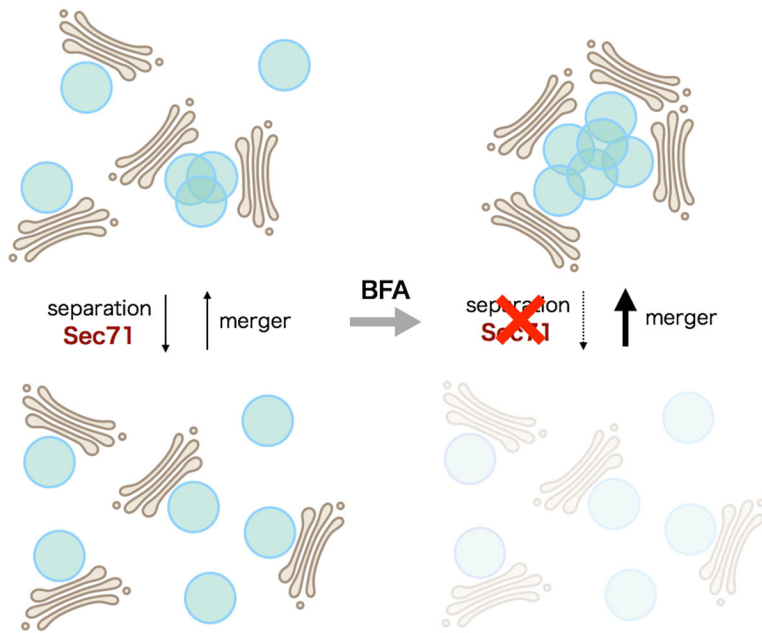
The pMT-hyg-V5::Sec71, pMT-hyg-V5::Sec71<sup>M717L</sup> and pMT-hyg-V5::Sec71<sup>F713Y</sup> plasmids were constructed using Gibson assembly-mediated site-directed mutagenesis. The fluorescent protein iRFP713

came from piRFP (Addgene #31857). The coding region of Sec71<sup>M717L</sup> was amplified from pMT-hyg-V5::Sec71<sup>M717L</sup> with primers Asc-GL3 and MT-Mlu-short, then cloned into pMT-hyg-V5::iRFP713::myc to produce pMT-hyg-V5::iRFP713::Sec71.

To construct CMV-ManII::EGFP and CMV-ST::EGFP, DNAs encoding amino acid residues 1–116 of mouse mannosidase II (Uniprot ID: P27046) and 1–43 of human sialyl-transferase (Uniprot ID: P15907) were amplified from plasmids Str-KDEL\_ManII-SBP-tagBFP (Addgene #65254) and Str-KDEL\_ST-SBP-tagBFP (AddGene #65266), deposited by Franck Perez (Boncompain et al., 2012), with primers Sac-ManII/ManII-B and Sac-ST/ST-Sal, respectively. These fragments were digested with SacI/BamHI and SacI/SalI, respectively, and then inserted into EGFP-golgi, replacing the *Galt* gene.

To construct pMT-GalT::EGFP, pMT-ST::EGFP and pMT-ManII::EGFP, NheI/NotI-digested fragments of CMV-GalT::EGFP, CMV-ST::EGFP and CMV-ManII::EGFP were inserted between SpeI and NotI sites of pMT-puro (Addgene plasmid #17923; deposited by David Sabatini).





**Fig. 8. BFA body formation model.** Golgi stacks and TGN/RE are shown in brown and blue, respectively. TGN/RE separations are driven by Sec71, and BFA inhibits Sec71 function. Without BFA, both separations and mergers of TGN/REs occur repeatedly, resulting in Golgi stack-associated TGN/RE being mostly separated in the steady state. After BFA addition, the separation of TGN/RE is greatly decreased, whereas mergers of TGN/RE occur normally, resulting in BFA body formation.

To construct pMT-GalT::EGFP-T2A-tdTomato::Rab11, the DNA fragment encoding GalT::EGFP was amplified from CMV-GalT::EGFP with primers msK-GalT-F and EGFP-dT2A and then with msK-GalT-F and dT2Aa. The DNA fragment coding tdTomato::Rab11 was amplified from pUAST-tdTomato-Rab11 WT (Addgene plasmid #53473; deposited by Matthew Scott), with primers dT2A-EGFP and Rab11-MT-Mlu, and then with dT2As and Rab11-MT-Mlu. To construct pMT-GalT::EGFP-T2A-tdTomato::Rab11, these two fragments were assembled between the KpnI and MluI sites of pMT-puro, using Gibson assembly. The plasmid pMT-GalT::EGFP-T2A-tdTomato::Rab6 was constructed similarly to the tdTomato-Rab6 fragment amplified from pMT-tdTomato::Rab6 with primers dT2A-EGFP and MT-Mlu-short.

## S2 cell culture

*Drosophila* S2 cells were cultured at 25°C in Schneider's medium (Gibco) supplemented with 10% inactivated fetal bovine serum and penicillin-streptomycin, as described in the standard protocol at DRSC/TRiP Functional Genomics Resources, Harvard University (<https://fgr.hms.harvard.edu/fly-cell-culture>).

## Knockdown by RNA interference in S2 cells

Pre-designed RNAi templates, DRSC07193 for *Garz* and DRSC01893 for *Sec71*, were selected from the UP-TORR Database (Hu et al., 2013). The templates for RNA synthesis were amplified from  $w^{1118}$  genomic DNA using KOD FX Neo (TOYOBO, Osaka, Japan) with primers garz-DRSC07193-F/garz-DRSC07193-R for *Garz*, and Sec71-DRSC01893-F/Sec71-DRSC01893-R for *Sec71*, respectively. T7 RNA polymerase promoters were added to both ends using 10 additional rounds of PCR using KOD Plus Neo (TOYOBO) with primers garz-DRSC07193-T7F/garz-DRSC07193-T7R for *Garz*, and Sec71-DRSC01893-T7F/Sec71-DRSC01893-T7R for *Sec71*, respectively. From these templates, double-stranded RNA was synthesized using the T7 High Yield RNA Synthesis Kit (NEB, Ipswich, MA, USA) and purified using RNA Clean & Concentrator-25 (Zymo Research, Irvine, CA, USA). Aliquots of 0.5 ml of 30–50% confluent S2 cells were transfected with 1 µg of dsRNA using 3 µl of FuGeneHD (Promega, Madison, WI, USA). At 18–24 h after transfection, the medium was replaced. Cells were incubated for 4–5 days after transfection, then subjected to immunostaining as described below.

## Establishment of genome-edited S2 cells with BFA-resistant mutant *Sec71*<sup>M717L</sup> or BFA-hypersensitive mutant *Sec71*<sup>F713Y</sup>

Designs of CRISPR and repair templates are shown in Fig. S1. A pair of CRISPR target sites was chosen to nest the 713Y and 717M mutations

between them. In addition to the desired substitutions, silent mutations were introduced on the proto-spacers to make the successfully edited allele CRISPR/Cas9-resistant. Approximately 200 bp of genomic sequences were added for homology-dependent repair on both sides. The repair templates were synthesized as 500-bp double-stranded DNA (dsDNA) fragments (gBlocks Gene Fragments, IDT), and PCR amplified using primers Sec71-GF14 and Sec71-GR6.

Chemically synthesized Alt-R CRISPR-Cas9 crRNAs (IDT, Coralville, IA, USA) were annealed with Alt-R CRISPR-Cas9 tracrRNAs (IDT, Coralville, IA, USA) to form duplexes. Then, 6 pmol of the duplex was incubated with 6 pmol of Alt-R S.p. We used Cas9 Nuclease V3 (IDT, Coralville, IA, USA) as the ribonucleoprotein (RNP) complex, according to the manufacturer's instructions. An aliquot of 0.5 ml of S2+ cells was transfected with 6 pmol of RNP and 250 ng of dsDNA repair template, using 3 µl of Lipofectamine RNAiMAX (Thermo Fisher Scientific, Waltham, MA, USA). To screen for BFA-resistant cells, *Sec71*<sup>M717L</sup>-knock-in cells were subcultured in medium containing 50 µM BFA for 30 days.

Because the null allele of *Sec71* showed strong lethality in the mosaic retina, a biallelic knockout of *Sec71* was expected to be lethal in S2 cells. Therefore, an additional knockout after knock-in by the same pair of CRISPR/Cas9 was expected to eliminate unedited alleles and enrich for the genome-edited allele. To enrich for BFA-hypersensitive cells, the *Sec71*<sup>F713Y</sup> knock-in was repeated three times; thereafter, the cells were knocked out four times by transfecting the RNP without repair template. To evaluate the efficiency of genome editing, the 849-bp fragment of genomic DNA was amplified using Sec71-GF5 and Sec71-GR4 primers, then digested with AluI, which digests only the unedited allele. As a result, the *Sec71*<sup>M717L</sup> knock-in reached 100% of the allele, with *Sec71*<sup>F713Y</sup> knock-in cells estimated to be 100%. These genome-edited cells were transfected with pMT-GalT::EGFP-T2A-tdTomato::Rab6 or pMT-GalT::EGFP-T2A-tdTomato::Rab11, using FuGeneHD (Promega). Transformants were selected by culturing with 2 µg/ml puromycin. After establishing stable transformants, efficiencies of genome editing were reconfirmed by PCR and AluI digestion.

## Immunostaining of S2 cells

*Drosophila* S2 cells were transiently transfected with pMT-ManII::EGFP, pMT-mTq2-Rab11, pMT-ST::EGFP, pMT-GalT::EGFP, pMK-V5-Sec71, or pMK-V5::Sec71<sup>E677K</sup>, pMK-V5-Garz, or pMK-V5::Garz<sup>E740K</sup> using FuGene HD (Promega). Expression was induced by adding 1 mM CuSO<sub>4</sub> at 2 days after transfection. For BFA treatment, the cells were incubated for 2 h with 50 µM BFA (Cayman Chemical Company, Ann Arbor, MI, USA). S2 cells previously transfected with pAc-mTq2-Rab11 or wild-type S2 cells

were transfected with double-stranded RNA as described above. Cells were fixed in 4% paraformaldehyde in PBS for 5 min at room temperature, rinsed three times for 2 min each in PBS, treated for 5 min in PBS containing 0.1% Triton X-100, and three 2 min rinses in PBS. Cells were incubated for 30 min in 20% bovine serum in PBS for blocking. Cells were incubated for 2 h in primary antibodies with 5% bovine serum in PBS. After three rinses for 2 min each in PBS, cells were incubated for 2 h with secondary antibodies. Cells were rinsed three times for 2 min each in PBS and then mounted in 50% glycerol in PBS containing 0.25% n-propyl gallate to inhibit fading. Primary antibodies were: mouse anti-V5 monoclonal antibody 6F5 (1:150) (WAKO Chemical #CTN3094, Osaka, Japan), rabbit anti-GM130 (1:300) (Abcam #ab30637, Cambridge, UK), guinea pig anti-Rab6 (1:300) (Iwanami et al., 2016), goat anti-Golgin245 (1:300) (DSHB) (Riedel et al., 2016), anti-Sec71 (1:300) (Wang et al., 2017), anti-AP1 $\gamma$  (1:2000) (Hirst et al., 2009), rat anti-p120 (1:15) (a gift from Dr Satoshi Goto, Rikkyo University, Tokyo, Japan) (Yamamoto-Hino et al., 2012), and rat anti-Rab11 (1:200) (Otsuka et al., 2019). Secondary antibodies were anti-mouse, anti-rabbit, and anti-guinea pig-IgG antibodies labeled with Alexa Fluor 488, 568 and 647 (1:300) (Life Technologies, Carlsbad, CA, USA). Sample images were recorded using FV1000 (PlanApo N 60 $\times$ 1.42 NA objective lens; Olympus, Tokyo, Japan) or FV3000 (PLAPON60XOSC2 1.4 NA objective lens; Olympus, Tokyo, Japan) confocal microscopes. To minimize bleedthrough, each color in double- or triple-stained samples was imaged sequentially. Images were processed in accordance with the Guidelines for Proper Digital Image Handling using Fiji, Affinity photo, and/or Adobe Photoshop CS3 (Adobe, San Jose, CA, USA).

For plotting immunostaining intensity across the Golgi stacks of BFA bodies, lines were drawn through each Golgi stack or BFA body, and intensities were measured using Fiji software and plotted using PLOT2 (micw.org). Representative plots are presented.

To quantify the proportions of S2 cells possessing BFA bodies, untreated and BFA-treated S2 cells were immunostained with anti-Rab6 and anti-GM130 antibodies, then observed under a FV3000 microscope with a 60 $\times$  objective lens. In these images, more than 250 cells for each condition were semi-automatically annotated for possessing BFA bodies or not; background staining by anti-Rab6 and anti-GM130 were used to define cells, with a concentrated Rab6 staining surrounded by GM130 staining scored as a BFA body. We performed three independent experiments for each condition, with means and standard deviations plotted in Fig. 2L.

### Live imaging of S2 cells by confocal microscopy

BFA-hypersensitive S2 cells were transfected with pMT-GalT-EGFP-T2A-tdTomato-Rab6 and selected with 2  $\mu$ g/ml puromycin for 2 weeks. Expression was induced by adding 0.5 mM CuSO<sub>4</sub> for 1 day. Cells were attached to a  $\mu$ -Slide 8-well chambered coverslip (ibidi, Martinsried, Germany) treated with poly-L-lysine (Merck KGaA, Darmstadt, Germany), and imaged on an FV3000 confocal microscope equipped with a PLAPON60XOSC2 1.4 NA objective lens (Olympus, Tokyo, Japan). For BFA treatment, the cells were incubated with 25  $\mu$ M BFA (Cayman Chemical Company). For each series, Z-stacks of three slices at 0.5- $\mu$ m intervals were taken every 1.5 min for 90 min. For quantification of the number of TGN merge and separation events, Z-stacks of 0.5  $\mu$ m, apart from five slices, were taken every 1 min for 60 min. TGN mergers and separations were counted in 16 untreated and BFA-treated cells, respectively, and plotted per cell (Fig. 4C). The ratio of TGN-merger to TGN separation within the same cell is plotted in Fig. 4D. Time-lapse series and movies were processed using ImageJ.

To show side views of merger and separation events, Z-stacks of 49 slices at 0.25- $\mu$ m intervals were taken every 20–60 s for 60 min. Volume-rendered images were generated using Fluorender.

### Live imaging of S2 cells by SCLIM

BFA-hypersensitive S2 cells stably transformed by *trans*-Golgi (GalT::EGFP) and TGN markers (tdTomato::Rab6) (see above) were further transfected with iRFP::Sec71 and selected with 200  $\mu$ g/ml hygromycin B for 2 weeks. BFA-hypersensitive S2 cells with GalT::EGFP, tdTomato::Rab6, and iRFP::Sec71 were inoculated on glass-based dishes (Iwaki,

Shizuoka, Japan). Cells were cultured for a day in phenol red-free medium to reduce background fluorescence, before observation by super-resolution confocal live imaging microscopy (SCLIM) (Kurokawa et al., 2013, 2019). For BFA treatment, cells were incubated for 2 h with 25  $\mu$ M BFA (Cayman Chemical Company). Z-stack images obtained by SCLIM were converted into 3D voxel data and processed by deconvolution with Volocity (Perkin Elmer, Waltham, MA, USA) using the theoretical point-spread function for spinning-disk confocal microscopy. Volume-rendered images were generated using Volocity or Fluorender, and time-lapse series and movies were processed using ImageJ.

### Electron microscopy imaging of GalT::APEX2::EGFP

*Drosophila* S2 cells were transformed by pMT-hyg-GalT-APEX2-EGFP using FuGENE HD (Promega), then selected in 200  $\mu$ g/ml hygromycin B for 3 weeks to establish a GalT-APEX2-EGFP stable transformant. Expression of GalT::APEX2::EGFP was induced by adding 0.5 mM CuSO<sub>4</sub> for 1 day. For Fig. 1F,G, the cells were incubated for 2 h with or without 50  $\mu$ M BFA (Cayman Chemical Company), then fixed in 2% glutaraldehyde, 2% paraformaldehyde and 2 mM CaCl<sub>2</sub> in 0.1 mM cacodylate buffer (pH 7.4) for 1 h on ice. For Fig. 2H,I, S2 cells stably transformed with GalT-APEX2-EGFP were transiently transfected with pMK-V5::Sec71<sup>E677K</sup> or pMK-V5::Garz<sup>E740K</sup> using FuGENE HD (Promega), then expression of both GalT::APEX2::EGFP and V5::Sec71<sup>E677K</sup>/V5::Garz<sup>E740K</sup> was induced by adding 0.5 mM CuSO<sub>4</sub> for 16–18 h. EM imaging of APEX2 was performed as described previously (Otsuka et al., 2019). The 70–90 nm sections were imaged using a JEM1400 transmission electron microscope (JEOL, Tokyo, Japan) operated at 80 kV; montage images were taken with a CCD camera system (JEOL, Tokyo, Japan).

### *Drosophila* stocks

Flies were grown at 20–25°C in standard cornmeal-glucose-agar-yeast medium, either in the laboratory with room light or in a 12L/12D incubator. The following fly stocks were used: Rh1-Gal4 (Chihiro Hama, Kyoto Sangyo University, Japan), longGMR-Gal4 (Bloomington Stock No. 8605, Bloomington, IN, USA; indicated as BL8605 in the following stocks), coin-FLP-Gal4 with UAS-2xEGFP (BL58751), UAS-V5::Sec71<sup>E677K</sup> (Fengwei Yu, Temasek Life Sciences Laboratory, Singapore), UAS-Syx5::myc (Richaud Burke, Monash University, Australia), UAS-GalT::ECFP (Satoh et al., 2005), UAS-Sec71-RNAi<sup>GLC01657</sup> (BL50539) and UAS-Sec71-RNAi<sup>HMS00357</sup> (BL32366). The females of ey-FLP, UAS-Dicer (BL24644) with second or third balancers were crossed with the males with UAS-Sec71-RNAi<sup>GLC01657</sup> or UAS-Sec71-RNAi<sup>HMS00357</sup>, respectively, and their male progeny were crossed to females with coin-FLP-Gal4 and UAS-2xEGFP to obtain mosaic retinas. The final cross for UAS-Sec71-RNAi<sup>HMS00357</sup> to obtain mosaic retinas was maintained at 18°C; otherwise, RNAi clones died during early stages of eye development.

### Immunostaining of fly retinas

Fixation and staining were performed as described previously (Fujii et al., 2020; Satoh and Ready, 2005). Primary antibodies were: rabbit anti-Rh1 (1:1000) (Satoh et al., 2005), mouse monoclonal anti-Na<sup>+</sup>K<sup>+</sup>-ATPase  $\alpha$  subunit (1:300 ascites) (DSHB, IA, USA), rabbit anti-GM130 (1:300) (Abcam #ab30637, Cambridge, UK), rabbit anti-MPPE (1:1000) (a gift from Junhai Han, Southeast University, Nanjing, China), rat anti-p120 (1:15) (a gift from Dr Satoshi Goto) (Yamamoto-Hino et al., 2012), rabbit anti-Rab6 (1:300) (Iwanami et al., 2016), guinea pig anti-Rab6 (1:300) (Iwanami et al., 2016), guinea pig anti-Sec71 (1:500) (Wang et al., 2017), rabbit anti-AP1 $\gamma$  (1:2000) (Hirst et al., 2009), Goat anti-Golgin245 (1:500) (DSHB) (Riedel et al., 2016), mouse anti-myc (1:15) (DSHB), mouse anti-Eys (1:15) (DSHB), guinea pig anti- $\alpha$ COP (1:150) (a gift from Dr Yoshihiro Inoue, Kyoto Sangyo University, Kyoto, Japan) (Kitazawa et al., 2012), rabbit anti-Cnx (1:300) (Satoh et al., 2015), rat anti-EMC3 (1:300) (Satoh et al., 2015), rat anti-Rab1 (1:250) (Otsuka et al., 2019), and rat anti-Rab11 (1:300) (Otsuka et al., 2019). Secondary antibodies were anti-mouse, anti-rabbit, anti-rat, anti-goat, and/or anti-guinea pig labeled with Alexa Fluor 488, 568 and 647 (1:300) (Life Technologies). Sample images were recorded using FV1000 (PlanApo N 60 $\times$ 1.42 NA objective lens; Olympus) or FV3000 (UPLSAPO60XS2 silicone-immersion 60x objective lens;



Olympus) confocal microscopes. To minimize bleed-through, each signal in double- or triple-stained samples was imaged sequentially. Images were processed in accordance with the Guidelines for Proper Digital Image Handling using Fiji, Affinity photo, and/or Adobe Photoshop CS3.

### Electron microscopy

Electron microscopy was performed as described previously (Satoh et al., 1997). Samples were observed under a JEM1400 EM (JEOL, Tokyo, Japan), and montages were prepared using a CCD camera system (JEOL). Phenotypes were investigated using sections at the depth at which multiple photoreceptor nuclei within ommatidia were observed.

### Acknowledgements

We thank Dr Junhai Han (Southeast University, Nanjing, China), Dr Satoshi Goto (Rikkyo University, Tokyo, Japan), Dr Robert Burke (Monash University, Victoria, Australia), Dr Fengwei Yu (Temasek Life Sciences Laboratory, Singapore), and Dr Stefan Luschign (University of Zurich, Zurich, Switzerland) for kindly providing reagents. We also thank the Bloomington *Drosophila* Stock Center and *Drosophila* Genomics and Genetic Resources. This work was made possible in part by software funded by the NIH: FluoRender: Visualization-based and Interactive Analysis for Multichannel Microscopy Data, 1 R01 EB023947, and National Institute of General Medical Sciences of the National Institutes of Health grant P41 GM103545.

### Competing interests

The authors declare no competing or financial interests.

### Author contributions

Conceptualization: T.S., A.K.S.; Methodology: S.F., K.K., T.S., A.K.S.; Formal analysis: S.F.; Investigation: S.F., K.K., T.T., R.I., A.T., T.S.; Data curation: S.F., T.S., A.K.S.; Writing - original draft: T.S., A.K.S.; Writing - review & editing: K.K., A.N.; Supervision: A.N., T.S., A.K.S.; Project administration: T.S., A.K.S.; Funding acquisition: K.K., A.N., T.S., A.K.S.

### Funding

This work was supported by Precursory Research for Embryonic Science and Technology grant 25-J4215, Japan Society for the Promotion of Science, KAKENHI grant 15K07050, Sumitomo Foundation for Basic Science Research Projects, Astellas Foundation for Research on Metabolic Disorders, and Female Researcher Joint Research Grant from Hiroshima University to A.S., KAKENHI grant 19K06566 to T.S., and KAKENHI grants 25221103, 17H06420, and 18H05275 to K.K. and A.N.

### Supplementary information

Supplementary information available online at <https://jcs.biologists.org/lookup/doi/10.1242/jcs.245571.supplemental>

### Peer review history

The peer review history is available online at <https://jcs.biologists.org/lookup/doi/10.1242/jcs.245571.viewer-comments.pdf>

### References

- Anders, N. and Jürgens, G. (2008). Large ARF guanine nucleotide exchange factors in membrane trafficking. *Cell. Mol. Life Sci.* **65**, 3433-3445. doi:10.1007/s00018-008-8227-7
- Armbruster, K. and Luschign, S. (2012). The *Drosophila* Sec7 domain guanine nucleotide exchange factor protein Gartenzwerg localizes at the cis-Golgi and is essential for epithelial tube expansion. *J. Cell Sci.* **125**, 1318-1328. doi:10.1242/jcs.096263
- Baluška, F., Hlavacka, A., Šamaj, J., Palme, K., Robinson, D. G., Matoh, T., McCurdy, D. W., Menzel, D. and Volkmann, D. (2002). F-actin-dependent endocytosis of cell wall pectins in meristematic root cells. Insights from brefeldin A-induced compartments. *Plant Physiol.* **130**, 422-431. doi:10.1104/pp.007526
- Boncompain, G., Divoux, S., Gareil, N., de Forges, H., Lescure, A., Latreche, L., Mercanti, V., Jollivet, F., Raposo, G. and Perez, F. (2012). Synchronization of secretory protein traffic in populations of cells. *Nat. Methods* **9**, 493-498. doi:10.1038/nmeth.1928
- Bosch, J. A., Tran, N. H. and Hariharan, I. K. (2015). CoinFLP: a system for efficient mosaic screening and for visualizing clonal boundaries in *Drosophila*. *Development* **142**, 597-606. doi:10.1242/dev.114603
- Casanova, J. E. (2007). Regulation of Arf activation: the Sec7 family of guanine nucleotide exchange factors. *Traffic* **8**, 1476-1485. doi:10.1111/j.1600-0854.2007.00634.x
- Chen, J., Call, G. B., Beyer, E., Bui, C., Cespedes, A., Chan, A., Chan, J., Chan, S., Chhabra, A., Dang, P. et al. (2005). Discovery-based science education: functional genomic dissection in *Drosophila* by undergraduate researchers. *PLoS Biol.* **3**, e59. doi:10.1371/journal.pbio.0030059
- Christis, C. and Munro, S. (2012). The small G protein Arf1 directs the trans-Golgi-specific targeting of the Arf1 exchange factors BIG1 and BIG2. *J. Cell Biol.* **196**, 327-335. doi:10.1083/jcb.201107115
- Cole, N. B., Smith, C. L., Sciaky, N., Terasaki, M., Edidin, M. and Lippincott-Schwartz, J. (1996). Diffusional mobility of Golgi proteins in membranes of living cells. *Science* **273**, 797-801. doi:10.1126/science.273.5276.797
- Cox, R., Mason-Gamer, R. J., Jackson, C. L. and Segev, N. (2004). Phylogenetic analysis of Sec7-domain-containing Arf nucleotide exchangers. *Mol. Biol. Cell* **15**, 1487-1505. doi:10.1091/mbc.e03-06-0443
- Donaldson, J. G., Lippincott-Schwartz, J., Bloom, G. S., Kreis, T. E. and Klausner, R. D. (1990). Dissociation of a 110-kD peripheral membrane protein from the Golgi apparatus is an early event in brefeldin A action. *J. Cell Biol.* **111**, 2295-2306. doi:10.1083/jcb.111.6.2295
- Dragwidge, J. M., Scholl, S., Schumacher, K. and Gendall, A. R. (2019). NHX-type Na<sup>+</sup>(K<sup>+</sup>)/H<sup>+</sup> antiporters are required for TGN/EE trafficking and endosomal ion homeostasis in *Arabidopsis thaliana*. *J. Cell Sci.* **132**, jcs226472. doi:10.1242/jcs.226472
- Fujii, S., Kurokawa, K., Inaba, R., Hiramatsu, N., Tago, T., Nakamura, Y., Nakano, A., Satoh, T. and Satoh, A. K. (2020). Recycling endosomes attach to the trans-side of Golgi stacks in *Drosophila* and mammalian cells. *J. Cell Sci.* **133**, jcs236935. doi:10.1242/jcs.236935
- Futter, C. E., Gibson, A., Allchin, E. H., Maxwell, S., Ruddock, L. J., Odorizzi, G., Domingo, D., Trowbridge, I. S. and Hopkins, C. R. (1998). In polarized MDCK cells basolateral vesicles arise from clathrin- $\gamma$ -adaptin-coated domains on endosomal tubules. *J. Cell Biol.* **141**, 611-623. doi:10.1083/jcb.141.3.611
- Geldner, N., Anders, N., Wolters, H., Keicher, J., Kornberger, W., Müller, P., Delbarre, A., Ueda, T., Nakano, A. and Jürgens, G. (2003). The Arabidopsis GNOM ARF-GEF mediates endosomal recycling, auxin transport, and auxin-dependent plant growth. *Cell* **112**, 219-230. doi:10.1016/S0092-8674(03)00003-5
- Goldenring, J. R. (2015). Recycling endosomes. *Curr. Opin. Cell Biol.* **35**, 117-122. doi:10.1016/j.cceb.2015.04.018
- Gosavi, P. and Gleeson, P. A. (2017). The function of the golgi ribbon structure - an enduring mystery unfolds! *BioEssays* **39**, 1700063. doi:10.1002/bies.201700063
- Hicke, L., Zanolari, B., Pypaert, M., Rohrer, J. and Riezman, H. (1997). Transport through the yeast endocytic pathway occurs through morphologically distinct compartments and requires an active secretory pathway and Sec18p/N-ethylmaleimide-sensitive fusion protein. *Mol. Biol. Cell* **8**, 13-31. doi:10.1091/mbc.8.1.13
- Hierro, A., Gershlick, D. C., Rojas, A. L. and Bonifacino, J. S. (2015). Formation of tubulovesicular carriers from endosomes and their fusion to the trans-Golgi network. *Int. Rev. Cell Mol. Biol.* **318**, 159-202. doi:10.1016/bs.ircmb.2015.05.005
- Hirst, J., Sahlender, D. A., Choma, M., Sinka, R., Harbour, M. E., Parkinson, M. P. and Robinson, M. S. (2009). Spatial and functional relationship of GGAs and AP-1 in *Drosophila* and HeLa cells. *Traffic* **10**, 1696-1710. doi:10.1111/j.1600-0854.2009.00983.x
- Hu, Y., Roesel, C., Flockhart, I., Perkins, L., Perrimon, N. and Mohr, S. E. (2013). UP-TORR: online tool for accurate and Up-to-Date annotation of RNAi Reagents. *Genetics* **195**, 37-45. doi:10.1534/genetics.113.151340
- Hunziker, W., Whitney, J. A. and Mellman, I. (1991). Selective inhibition of transcytosis by brefeldin A in MDCK cells. *Cell* **67**, 617-627. doi:10.1016/0092-8674(91)90535-7
- Husain, N., Pellikka, M., Hong, H., Klimentova, T., Choe, K.-M., Clandinin, T. R. and Teppas, U. (2006). The agrin/perlecan-related protein eyes shut is essential for epithelial lumen formation in the *Drosophila* retina. *Dev. Cell* **11**, 483-493. doi:10.1016/j.devcel.2006.08.012
- Ishizaki, R., Shin, H.-W., Mitsuhashi, H. and Nakayama, K. (2008). Redundant roles of BIG2 and BIG1, guanine-nucleotide exchange factors for ADP-ribosylation factors in membrane traffic between the trans-Golgi network and endosomes. *Mol. Biol. Cell* **19**, 2650-2660. doi:10.1091/mbc.e07-10-1067
- Ito, Y., Uemura, T., Shoda, K., Fujimoto, M., Ueda, T. and Nakano, A. (2012). cis-Golgi proteins accumulate near the ER exit sites and act as the scaffold for Golgi regeneration after brefeldin A treatment in tobacco BY-2 cells. *Mol. Biol. Cell* **23**, 3203-3214. doi:10.1091/mbc.e12-01-0034
- Ito, Y., Toyooka, K., Fujimoto, M., Ueda, T., Uemura, T. and Nakano, A. (2017). The trans-Golgi Network and the Golgi Stacks Behave Independently During Regeneration After Brefeldin A Treatment in Tobacco BY-2 Cells. *Plant Cell Physiol.* **58**, 811-821. doi:10.1093/pcp/pcx028
- Ito, Y., Uemura, T. and Nakano, A. (2018). The Golgi entry core compartment functions as a COPII-independent scaffold for ER-to-Golgi transport in plant cells. *J. Cell Sci.* **131**, jcs203893. doi:10.1242/jcs.203893
- Iwanami, N., Nakamura, Y., Satoh, T., Liu, Z. and Satoh, A. K. (2016). Rab6 is required for multiple apical transport pathways but not the basolateral transport pathway in *Drosophila* photoreceptors. *PLoS Genet.* **12**, e1005828. doi:10.1371/journal.pgen.1005828
- Jackson, C. L. (2018). Activators and effectors of the small G protein Arf1 in regulation of Golgi dynamics during the cell division cycle. *Front. Cell Dev. Biol.* **6**, 29. doi:10.3389/fcell.2018.00029

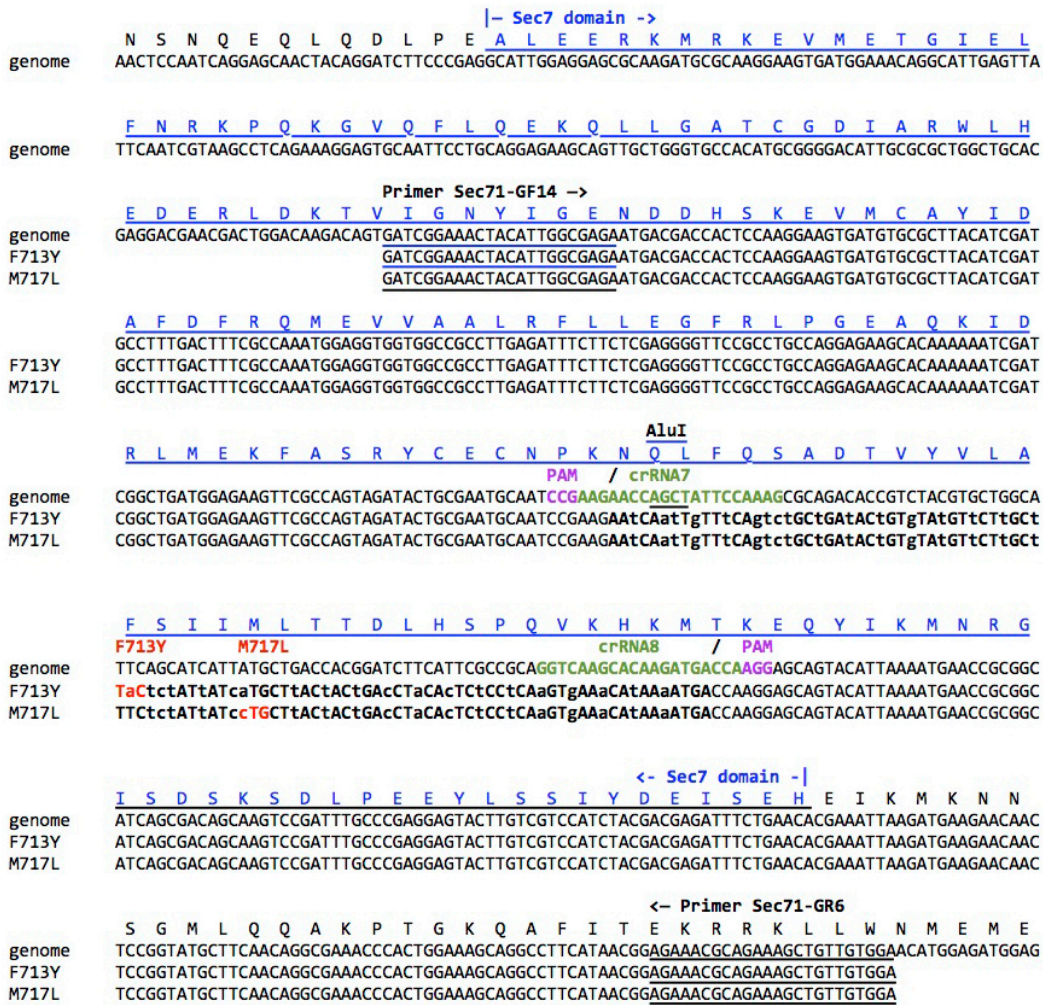
- Jiang, S., Rhee, S. W., Gleeson, P. A. and Storrie, B. (2006). Capacity of the Golgi apparatus for cargo transport prior to complete assembly. *Mol. Biol. Cell* **17**, 4105-4117. doi:10.1091/mbc.e05-12-1112
- Kang, B.-H., Nielsen, E., Preuss, M. L., Mastronarde, D. and Staehelin, L. A. (2011). Electron tomography of RabA4b- and PI-4K $\beta$ -labeled trans Golgi network compartments in Arabidopsis. *Traffic* **12**, 313-329. doi:10.1111/j.1600-0854.2010.01146.x
- Kienzle, C. and von Blume, J. (2014). Secretory cargo sorting at the trans-Golgi network. *Trends Cell Biol.* **24**, 584-593. doi:10.1016/j.tcb.2014.04.007
- Kitazawa, D., Yamaguchi, M., Mori, H. and Inoue, Y. H. (2012). COPI-mediated membrane trafficking is required for cytokinesis in *Drosophila* male meiotic divisions. *J. Cell Sci.* **125**, 3649-3660. doi:10.1242/jcs.103317
- Klumperman, J. (2011). Architecture of the mammalian Golgi. *Cold Spring Harb. Perspect. Biol.* **3**, a005181. doi:10.1101/cshperspect.a005181
- Kondylis, V. and Rabouille, C. (2003). A novel role for dp115 in the organization of tER sites in *Drosophila*. *J. Cell Biol.* **162**, 185-198. doi:10.1083/jcb.200301136
- Kondylis, V. and Rabouille, C. (2009). The Golgi apparatus: lessons from *Drosophila*. *FEBS Lett.* **583**, 3827-3838. doi:10.1016/j.febslet.2009.09.048
- Kondylis, V., van Nispen tot Pannerden, H. E., Herpers, B., Friggi-Grelin, F. and Rabouille, C. (2007). The golgi comprises a paired stack that is separated at G2 by modulation of the actin cytoskeleton through Abi and Scar/WAVE. *Dev. Cell* **12**, 901-915. doi:10.1016/j.devcel.2007.03.008
- Kurokawa, K., Ishii, M., Suda, Y., Ichihara, A. and Nakano, A. (2013). Live cell visualization of Golgi membrane dynamics by super-resolution confocal live imaging microscopy. *Methods Cell Biol.* **118**, 235-242. doi:10.1016/B978-0-12-417164-0.00014-8
- Kurokawa, K., Osakada, H., Kojidani, T., Waga, M., Suda, Y., Asakawa, H., Haraguchi, T. and Nakano, A. (2019). Visualization of secretory cargo transport within the Golgi apparatus. *J. Cell Biol.* **218**, 1602-1618. doi:10.1083/jcb.201807194
- Laffafian, A. and Tepass, U. (2019). Identification of genes required for apical protein trafficking in *Drosophila* photoreceptor cells. *G3 (Bethesda)* **9**, 4007-4017. doi:10.1534/g3.119.400635
- Langhans, M., Förster, S., Helmchen, G. and Robinson, D. G. (2011). Differential effects of the brefeldin A analogue (6R)-hydroxy-BFA in tobacco and Arabidopsis. *J. Exp. Bot.* **62**, 2949-2957. doi:10.1093/jxb/err007
- Lippincott-Schwartz, J. and Liu, W. (2006). Insights into COPI coat assembly and function in living cells. *Trends Cell Biol.* **16**, e1-e4. doi:10.1016/j.tcb.2006.08.008
- Lippincott-Schwartz, J., Yuan, L. C., Bonifacino, J. S. and Klausner, R. D. (1989). Rapid redistribution of Golgi proteins into the ER in cells treated with brefeldin A: evidence for membrane cycling from Golgi to ER. *Cell* **56**, 801-813. doi:10.1016/0092-8674(89)90685-5
- Lippincott-Schwartz, J., Yuan, L., Tipper, C., Amherdt, M., Orci, L. and Klausner, R. D. (1991). Brefeldin A's effects on endosomes, lysosomes, and the TGN suggest a general mechanism for regulating organelle structure and membrane traffic. *Cell* **67**, 601-616. doi:10.1016/0092-8674(91)90534-6
- Luini, A. and Parashuraman, S. (2016). Signaling at the Golgi: sensing and controlling the membrane fluxes. *Curr. Opin. Cell Biol.* **39**, 37-42. doi:10.1016/j.ccb.2016.01.014
- Makaraci, P. and Kim, K. (2018). trans-Golgi network-bound cargo traffic. *Eur. J. Cell Biol.* **97**, 137-149. doi:10.1016/j.ejcb.2018.01.003
- Martell, J. D., Deerinck, T. J., Lam, S. S., Ellisman, M. H. and Ting, A. Y. (2017). Electron microscopy using the genetically encoded APEX2 tag in cultured mammalian cells. *Nat. Protoc.* **12**, 1792-1816. doi:10.1038/nprot.2017.065
- Mayor, S., Presley, J. F. and Maxfield, F. R. (1993). Sorting of membrane components from endosomes and subsequent recycling to the cell surface occurs by a bulk flow process. *J. Cell Biol.* **121**, 1257-1269. doi:10.1083/jcb.121.6.1257
- McDonold, C. M. and Fromme, J. C. (2014). Four GTPases differentially regulate the Sec7 Arf-GEF to direct traffic at the trans-golgi network. *Dev. Cell* **30**, 759-767. doi:10.1016/j.devcel.2014.07.016
- Misaki, R., Morimatsu, M., Uemura, T., Waguri, S., Miyoshi, E., Taniguchi, N., Matsuda, M. and Taguchi, T. (2010). Palmitoylated Ras proteins traffic through recycling endosomes to the plasma membrane during exocytosis. *J. Cell Biol.* **191**, 23-29. doi:10.1083/jcb.200911143
- Norgate, M., Southon, A., Greenough, M., Cater, M., Farlow, A., Batterham, P., Bush, A. I., Subramaniam, V. N., Burke, R. and Camakaris, J. (2010). Syntaxin 5 is required for copper homeostasis in *Drosophila* and mammals. *PLoS ONE* **5**, e14303. doi:10.1371/journal.pone.0014303
- Orci, L., Tagaya, M., Amherdt, M., Perrelet, A., Donaldson, J. G., Lippincott-Schwartz, J., Klausner, R. D. and Rothman, J. E. (1991). Brefeldin A, a drug that blocks secretion, prevents the assembly of non-clathrin-coated buds on Golgi cisternae. *Cell* **64**, 1183-1195. doi:10.1016/0092-8674(91)90273-2
- Otsuka, Y., Satoh, T., Nakayama, N., Inaba, R., Yamashita, H. and Satoh, A. K. (2019). Parcas is the predominant Rab11-GEF for rhodopsin transport in *Drosophila* photoreceptors. *J. Cell Sci.* **132**, jcs231431. doi:10.1242/jcs.231431
- Papanikou, E. and Glick, B. S. (2014). Golgi compartmentation and identity. *Curr. Opin. Cell Biol.* **29**, 74-81. doi:10.1016/j.ccb.2014.04.010
- Peyroche, A., Antonny, B., Robineau, S., Acker, J., Cherfils, J. and Jackson, C. L. (1999). Brefeldin A acts to stabilize an abortive ARF-GDP-Sec7 domain protein complex: involvement of specific residues of the Sec7 domain. *Mol. Cell* **3**, 275-285. doi:10.1016/S1097-2765(00)80455-4
- Peyroche, A., Paris, S. and Jackson, C. L. (1996). Nucleotide exchange on ARF mediated by yeast Gea1 protein. *Nature* **384**, 479-481. doi:10.1038/384479a0
- Peyroche, A., Courbeyrette, R., Rambourg, A. and Jackson, C. L. (2001). The ARF exchange factors Gea1p and Gea2p regulate Golgi structure and function in yeast. *J. Cell Sci.* **114**, 2241-2253.
- Renault, L., Guibert, B. and Cherfils, J. (2003). Structural snapshots of the mechanism and inhibition of a guanine nucleotide exchange factor. *Nature* **426**, 525-530. doi:10.1038/nature02197
- Riedel, F., Gillingham, A. K., Rosa-Ferreira, C., Galindo, A. and Munro, S. (2016). An antibody toolkit for the study of membrane traffic in *Drosophila melanogaster*. *Biol. Open* **5**, 987-992. doi:10.1242/bio.018937
- Robinson, D. G., Langhans, M., Saint-Jore-Dupas, C. and Hawes, C. (2008). BFA effects are tissue and not just plant specific. *Trends Plant Sci.* **13**, 405-408. doi:10.1016/j.tplants.2008.05.010
- Rogers, G. C., Rusan, N. M., Peifer, M. and Rogers, S. L. (2008). A multicomponent assembly pathway contributes to the formation of acentrosomal microtubule arrays in interphase *Drosophila* cells. *Mol. Biol. Cell* **19**, 3163-3178. doi:10.1091/mbc.e07-10-1069
- Rosenbaum, E. E., Hardie, R. C. and Colley, N. J. (2006). Calnexin is essential for rhodopsin maturation, Ca<sup>2+</sup> regulation, and photoreceptor cell survival. *Neuron* **49**, 229-241. doi:10.1016/j.neuron.2005.12.011
- Rusan, N. M. and Rogers, G. C. (2009). Centrosome function: sometimes less is more. *Traffic* **10**, 472-481. doi:10.1111/j.1600-0854.2009.00880.x
- Saraste, J. and Prydz, K. (2019). A new look at the functional organization of the Golgi ribbon. *Front. Cell Dev. Biol.* **7**, 171. doi:10.3389/fcell.2019.00171
- Sata, M., Donaldson, J. G., Moss, J. and Vaughan, M. (1998). Brefeldin A-inhibited guanine nucleotide-exchange activity of Sec7 domain from yeast Sec7 with yeast and mammalian ADP ribosylation factors. *Proc. Natl. Acad. Sci. USA* **95**, 4204-4208. doi:10.1073/pnas.95.8.4204
- Satoh, A. K. and Ready, D. F. (2005). Arrestin1 mediates light-dependent rhodopsin endocytosis and cell survival. *Curr. Biol.* **15**, 1722-1733. doi:10.1016/j.cub.2005.08.064
- Satoh, A., Tokunaga, F., Kawamura, S. and Ozaki, K. (1997). In situ inhibition of vesicle transport and protein processing in the dominant negative Rab1 mutant of *Drosophila*. *J. Cell Sci.* **110**, 2943-2953.
- Satoh, A. K., O'Tousa, J. E., Ozaki, K. and Ready, D. F. (2005). Rab11 mediates post-Golgi trafficking of rhodopsin to the photosensitive apical membrane of *Drosophila* photoreceptors. *Development* **132**, 1487-1497. doi:10.1242/dev.01704
- Satoh, T., Ohba, A., Liu, Z., Inagaki, T. and Satoh, A. K. (2015). dPob/EMC is essential for biosynthesis of rhodopsin and other multi-pass membrane proteins in *Drosophila* photoreceptors. *eLife* **4**, e06306. doi:10.7554/eLife.06306
- Sciaky, N., Presley, J., Smith, C., Zaal, K. J. M., Cole, N., Moreira, J. E., Terasaki, M., Siggia, E. and Lippincott-Schwartz, J. (1997). Golgi tubule traffic and the effects of brefeldin A visualized in living cells. *J. Cell Biol.* **139**, 1137-1155. doi:10.1083/jcb.139.5.1137
- Shin, H.-W. and Nakayama, K. (2004). Guanine nucleotide-exchange factors for arf GTPases: their diverse functions in membrane traffic. *J. Biochem.* **136**, 761-767. doi:10.1093/jb/mvh185
- Sisson, J. C., Field, C., Ventura, R., Royou, A. and Sullivan, W. (2000). Lava lamp, a novel peripheral golgi protein, is required for *Drosophila melanogaster* cellularization. *J. Cell Biol.* **151**, 905-918. doi:10.1083/jcb.151.4.905
- Teh, O.-K. and Moore, I. (2007). An ARF-GEF acting at the Golgi and in selective endocytosis in polarized plant cells. *Nature* **448**, 493-496. doi:10.1038/nature06023
- Uemura, T. and Nakano, A. (2013). Plant TGNs: dynamics and physiological functions. *Histochem. Cell Biol.* **140**, 341-345. doi:10.1007/s00418-013-1116-7
- Uemura, T., Suda, Y., Ueda, T. and Nakano, A. (2014). Dynamic behavior of the trans-golgi network in root tissues of Arabidopsis revealed by super-resolution live imaging. *Plant Cell Physiol.* **55**, 694-703. doi:10.1093/pcp/pcu010
- Uemura, T., Nakano, R. T., Takagi, J., Wang, Y., Kramer, K., Finkemeier, I., Nakagami, H., Tsuda, K., Ueda, T., Schulze-Lefert, P. et al. (2019). A golgi-released subpopulation of the trans-golgi network mediates protein secretion in Arabidopsis. *Plant Physiol.* **179**, 519-532. doi:10.1104/pp.18.01228
- Verheije, M. H., Raaben, M., Mari, M., Te Lintelo, E. G., Reggiori, F., van Kuppeveld, F. J. M., Rottier, P. J. M. and de Haan, C. A. M. (2008). Mouse hepatitis coronavirus RNA replication depends on GBF1-mediated ARF1 activation. *PLoS Pathog.* **4**, e1000088. doi:10.1371/journal.ppat.1000088
- Viaud, J., Zeghouf, M., Barelli, H., Zeeh, J.-C., Padilla, A., Guibert, B., Chardin, P., Royer, C. A., Cherfils, J. and Chavanieu, A. (2007). Structure-based discovery of an inhibitor of Arf activation by Sec7 domains through targeting of protein-protein complexes. *Proc. Natl. Acad. Sci. USA* **104**, 10370-10375. doi:10.1073/pnas.0700773104
- Viotti, C., Bubeck, J., Stierhof, Y.-D., Krebs, M., Langhans, M., van den Berg, W., van Dongen, W., Richter, S., Geldner, N., Takano, J. et al. (2010). Endocytic and secretory traffic in Arabidopsis merge in the trans-Golgi network/early endosome, an independent and highly dynamic organelle. *Plant Cell* **22**, 1344-1357. doi:10.1105/tpc.109.072637



- Walenta, J. H., Didier, A. J., Liu, X. and Krämer, H.** (2001). The Golgi-associated hook3 protein is a member of a novel family of microtubule-binding proteins. *J. Cell Biol.* **152**, 923-934. doi:10.1083/jcb.152.5.923
- Wang, S., Meyer, H., Ochoa-Espinosa, A., Buchwald, U., Onel, S., Altenhein, B., Heinisch, J. J., Affolter, M. and Paululat, A.** (2012). GBF1 (Gartenzwerg)-dependent secretion is required for *Drosophila* tubulogenesis. *J. Cell Sci.* **125**, 461-472. doi:10.1242/jcs.092551
- Wang, Y., Zhang, H., Shi, M., Liou, Y.-C., Lu, L. and Yu, F.** (2017). Sec71 functions as a GEF for the small GTPase Arf1 to govern dendrite pruning of *Drosophila* sensory neurons. *Development* **144**, 1851-1862. doi:10.1242/dev.146175
- Wei, J.-H. and Seemann, J.** (2017). Golgi ribbon disassembly during mitosis, differentiation and disease progression. *Curr. Opin. Cell Biol.* **47**, 43-51. doi:10.1016/j.cob.2017.03.008
- Wood, S. A., Park, J. E. and Brown, W. J.** (1991). Brefeldin A causes a microtubule-mediated fusion of the trans-Golgi network and early endosomes. *Cell* **67**, 591-600. doi:10.1016/0092-8674(91)90533-5
- Wright, J., Kahn, R. A. and Sztul, E.** (2014). Regulating the large Sec7 ARF guanine nucleotide exchange factors: the when, where and how of activation. *Cell. Mol. Life Sci.* **71**, 3419-3438. doi:10.1007/s00018-014-1602-7
- Xu, H., Boulianne, G. L. and Trimble, W. S.** (2002). *Drosophila* syntaxin 16 is a Q-SNARE implicated in Golgi dynamics. *J. Cell Sci.* **115**, 4447-4455. doi:10.1242/jcs.00139
- Yadav, S. and Linstedt, A. D.** (2011). Golgi positioning. *Cold Spring Harb. Perspect. Biol.* **3**, a005322. doi:10.1101/cshperspect.a005322
- Yamamoto-Hino, M., Abe, M., Shibano, T., Setoguchi, Y., Awano, W., Ueda, R., Okano, H. and Goto, S.** (2012). Cisterna-specific localization of glycosylation-related proteins to the Golgi apparatus. *Cell Struct. Funct.* **37**, 55-63. doi:10.1247/csf.11037
- Yamashiro, D. J. and Maxfield, F. R.** (1987). Acidification of morphologically distinct endosomes in mutant and wild-type Chinese hamster ovary cells. *J. Cell Biol.* **105**, 2723-2733. doi:10.1083/jcb.105.6.2723
- Yasuhara, H. and Shibaoka, H.** (2000). Inhibition of cell-plate formation by brefeldin A inhibited the depolymerization of microtubules in the central region of the phragmoplast. *Plant Cell Physiol.* **41**, 300-310. doi:10.1093/pcp/41.3.300
- Yasuhara, H., Sonobe, S. and Shibaoka, H.** (1995). Effects of brefeldin A on the formation of the cell plate in tobacco BY-2 cells. *Eur. J. Cell Biol.* **66**, 274-281.
- Zeeh, J.-C., Zeghouf, M., Grauffel, C., Guibert, B., Martin, E., Dejaegere, A. and Cherfils, J.** (2006). Dual specificity of the interfacial inhibitor brefeldin a for arf proteins and sec7 domains. *J. Biol. Chem.* **281**, 11805-11814. doi:10.1074/jbc.M600149200

## Supplemental information

### Fujii S et al., Figure S1

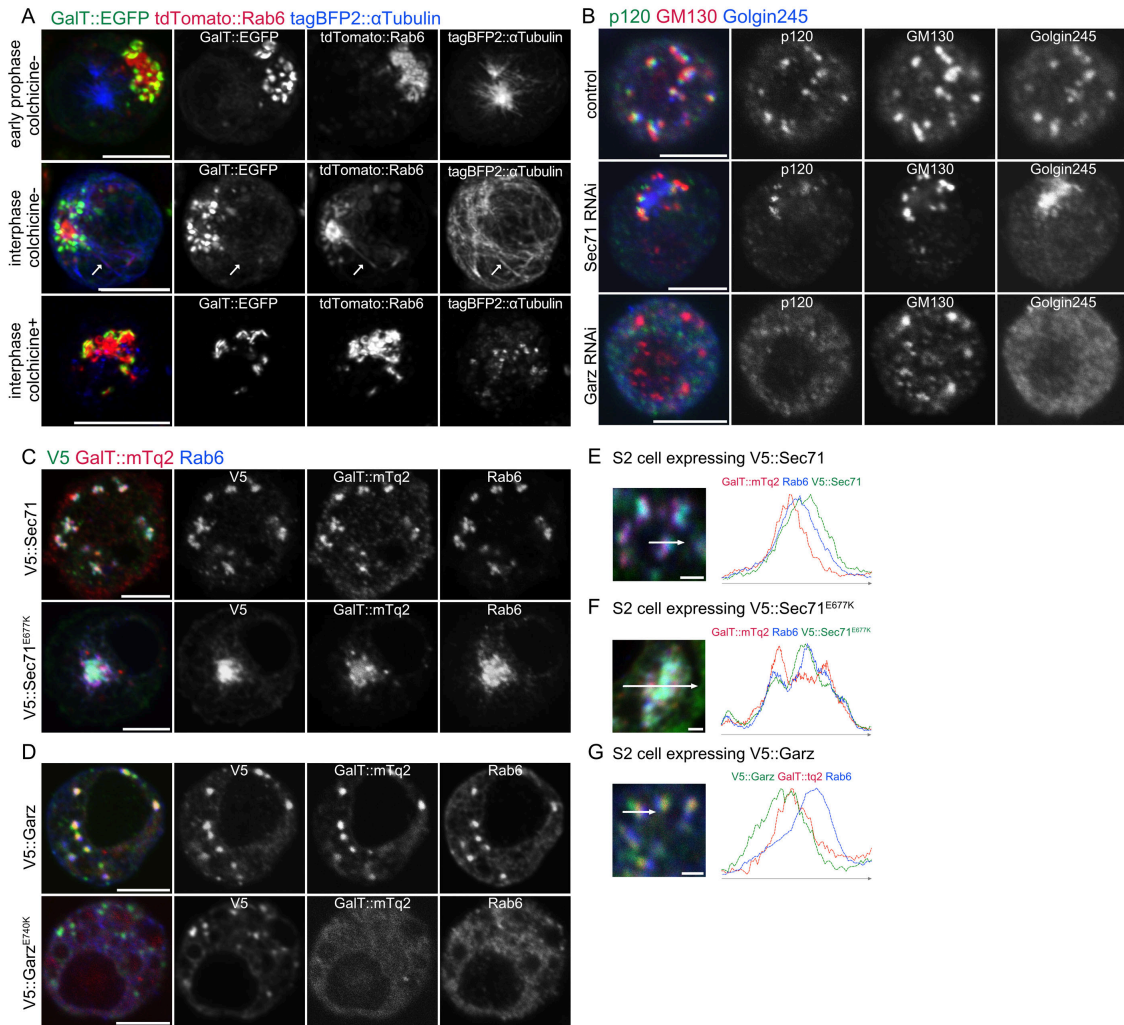


**Fig S1. Schematic of Sec71 genome-editing repair templates.**

Sec7-domain (blue), sequences (black), and mutations (lower case) to be knocked in, 713Y and M717L mutations (red), protospacers (green), PAM (violet), and predicted breakpoints (/).



Fujii S et al., Figure S2

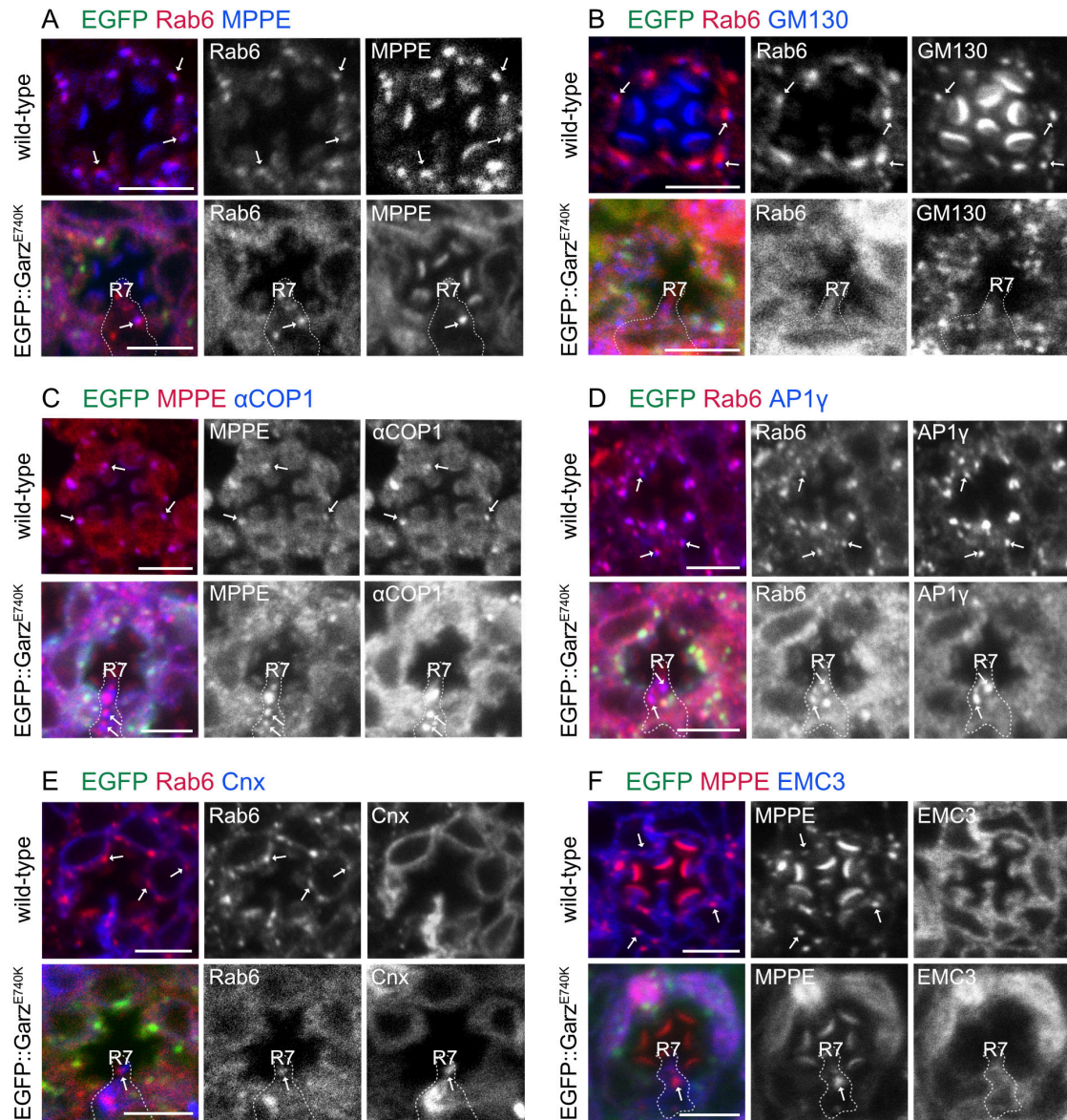


**Fig S2. Effects of Sec71 or Garz impairment on the distribution of Golgi enzymes in S2 cells.**

(A) Sec71<sup>F713Y</sup> cells expressing tagBFP2:: $\alpha$ Tubulin (blue), GalT::EGFP (green), and tdTomato::Rab6 (red) were treated with 25  $\mu$ M BFA for 2 h, and fixed. Z-series stacks at 0.3- $\mu$ m intervals were obtained by FV3000 confocal microscopy. Projected images by maximum intensity are presented. The cell shown in the bottom row was incubated with 30  $\mu$ M colchicine and 25  $\mu$ M BFA. (B) Immunostaining of cells without (upper panels), or with transfection of double-stranded RNA against Sec71 (panels in middle row), or Garz (lower panel), by anti-p120 (green), anti-GM130 (red) and anti-Golgin245 (blue) antibodies. (C) Immunostaining of cells expressing V5::Sec71 (upper panels) or V5::Sec71<sup>E677K</sup> (lower panels) together with GalT::mTq2 (red) by anti-V5 (green) and anti-Rab6 (blue) antibodies. (D) Immunostaining of S2 cell expressing V5::Garz (upper panels) or V5::Garz<sup>E740K</sup> (lower panels) together with GalT::mTq2 (red) by anti-V5 (green) and anti-Rab6 (blue) antibodies. (E, F, G) Left, immunostaining of cells expressing V5::Sec71 (E), V5::Sec71<sup>E677K</sup> (F) or V5::Garz (G) together with GalT::mTq2 (red) by anti-V5 (green) and anti-Rab6 (blue) antibodies. Right, plots of signal intensities from images on the left. Signal intensities were measured along the 1.5- $\mu$ m (E, G) or 5- $\mu$ m (F) arrows in insets; graphs show the overlap between channels. Scale bars: 5  $\mu$ m (A–D) and 1  $\mu$ m (E–G).



### Fujii et al., Figure S3



**Fig S3. Golgi resident proteins, except GM130, are localized to ER in *Garz*<sup>E740K</sup>-expressing photoreceptors.**

Immunostaining of retinas from Rh1-Gal4/+ (upper panels) and Rh1-Gal4/ UAS-EGFP::*Garz*<sup>E740K</sup> (lower panels) late-pupal flies. EGFP::*Garz*<sup>E740K</sup> is expressed in R1-6 peripheral photoreceptors. Green represents the EGFP signal. Anti-Rab6 (red) and anti-MPPE (blue) antibodies (A). Anti-Rab6 (red) and anti-GM130 (blue) antibodies (B). Anti-Rab6 (red) and anti- $\alpha$ COPI (blue) antibodies (C). Anti-Rab6 (red) and anti-AP1 $\gamma$  (blue) antibodies (D). Anti-Rab6 (red) and anti- $\alpha$ Cnx (blue) antibodies (E). Anti-MPPE (red) and anti-EMC3 (blue) antibodies (F). Arrows indicate Golgi stacks in wild-type cells. Scale bar: 5  $\mu$ m (A–F).





**Movie 1. S2 cells expressing GalT::EGFP (green) and tdTomato::Rab6 (red) with or without BFA.**

Each frame represents the projection of three slices at 0.5  $\mu\text{m}$  intervals, taken every 90 or 60 seconds without or with 25  $\mu\text{M}$  BFA, collected by FV3000 confocal microscopy.

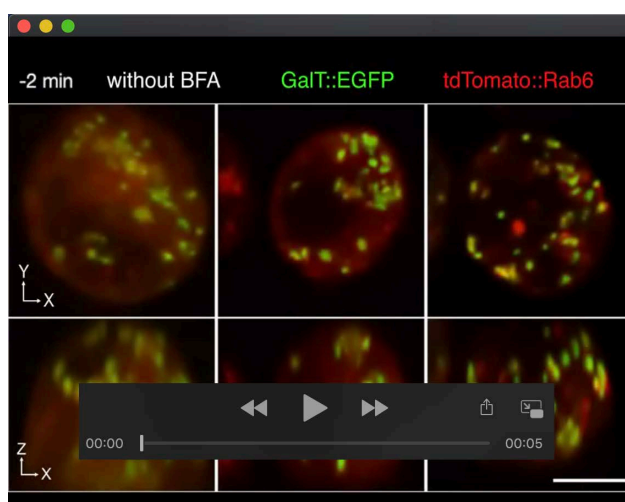
Arrows indicate mergers and arrowheads indicate separation of TGNs. The images in Fig. 4A and B were taken from this movie.

Scale bar: 5  $\mu\text{m}$ .



**Movie 2. S2 cells expressing GalT::EGFP (green) and tdTomato::Rab6 (red) without BFA.**

For two Sec71<sup>F713Y</sup> S2 cells expressing GalT::EGFP and tdTomato::Rab6 without BFA, volumes of 49 slices of 0.25- $\mu\text{m}$  thick images with 15 s interval, collected by FV3000 confocal microscopy. The area sparse with Golgi stacks were cropped and volume-rendered using Fluorender to generate images from the views of three axis. Arrows indicate mergers and arrowheads indicate separations of TGNs, respectively. Scale bar: 5  $\mu\text{m}$ .



**Movie 3. S2 cells expressing GalT::EGFP (green) and tdTomato::Rab6 (red) after BFA treatment.**

Three Sec71<sup>F713Y</sup> S2 cells (left, middle, and right) before and after 25 μM BFA addition are shown as Z-projections (upper row) and side views (lower row). Each frame represents the z-projected image of 49 slices of 0.25 μm thick images taken every 60 s for 59 min, collected by FV3000 confocal microscopy. Scale bar: 5 μm.



**Movie 4. S2 cells expressing GalT::EGFP (green), tdTomato::Rab6 (red), and iRFP713::Sec71.**

Each frame represents volume-rendered 3D images captured at 1.8-s intervals, collected by SCLIM. Without BFA, 10 or 79 min after 25 μM BFA addition are presented. Grid: 1 μm.



Table S1. List of Primers used in this study

Cloning of Sec71 cDNA	
Sec71-GF1	AGGGAAATCCGAAGCAGTGGTAGTAGG
Sec71-GR2	CTACGATTTGAGGGGATTTTCGGGTGG
GL3-Sec71	ggaggaggttctggtggtgtCACAACAACCTCCACAAA AACCAAGG
Sec71-MK-Sp	gatcttcatggtcgactagaATTATTTAACGCTCATTATA TTGAATACTGG
B-MK-V5	ctaaaggggggatctcgagGccaccATGGGTAAGCCTAT CCCT
GL3-R	accaccaccagaacctctctctcc
MT-Mlu-Short	GATGGTGATGATGaccggtacgCG
Cloning of garz cDNA	
garz-GF1	gagtgcctaaacatatgcgaaggtc
garz-GR1	aagacggtcaggtggtttgattgaa
GL3-garz	ggaggaggttctggtggtgtGCGCTTCCAGGCAACGG CAT
garz-MK-Sp	gatcttcatggtcgactagaATTACTGCTGGCCGTAGAG CAGTTC
Sequencing of Sec71 cDNA	
Sec71-GF3	CAGCAAGAACCTGGTCAATC
Sec71-GF4	CGGCTAAGTTTACGCACATC
Sec71-GF5	AAGTGCATGGTAGAGTGGAG
Sec71-GF6	CACGGATGGCAATTATCTGG
Sec71-GF7	gatctgtacaagcgtcagttcg
Sec71-GF8	GTGTGTTTCAGCAGAGTAACG
Sec71-GF9	AATGTTTCATCGTGCGTGC
Sec71-GF10	ATCTGAGAACCCGCCAGC

Sec71-GF11	CAAGATGACCAAGGAGCAG
Sec71-GF12	AGCCTCAGAAAGGAGTGCAATTCC
Sec71-GF13	CAGTGGTCACGCATTTGGC
Sec71-GF14	GATCGGAAACTACATTGGCGAGA
Sec71-GF15	TCGCCAGTAGATACTGCGAATG
Sec71-GR3	GGCGAACTCTGACAGGCACTTG
Sec71-GR4	CAACAGCTTTCTGCGTTTCTCCGT
Sec71-GR5	CGCGGTTTCATTTAATGTACTGCTC
Sec71-GR6	TCCACAACAGCTTTCTGCGTTTCT
Sequencing of garz cDNA	
garz-GF3	AGAATGACTCGCTGCTGAAG
garz-GF4	GTATGAGGGCTTGCTGGAG
garz-GF5	CGACTACGACTACAATCTGGC
garz-GF6	TTTGCGTGGTCTAAACGG
garz-GF7	G TTCACGACTACGGCGATTG
garz-GF8	ATGCGAAACGAAGA ACTGTG
garz-GF9	GCACACATCACGCCTTAC
garz-GF10	ATTATGTCTAAGGTGTTCTCTGC
garz-GF11	GTGAATGAGGCAACTGCTG
garz-GF12	GTGAATGAGGCAACTGCTG
garz-GF13	GCCGAGGCTGAGTGA ACTG
Site-directed mutagenesis	
Sec71-E677K-R	TTTTGTGCTTtTCCTGGCAGGCGGAACCCCT CGAG
Sec71-E677K-F	TCCGCCTGCCAGGAaAAGCACAAAAATCG ATCGGCTGA
garz-E740K-F	GATTGCCCGGAAAGGCTCCATTGATCTTTTT GGTGCTG
garz-E740K-R	ATCAATGGAGCCTTTCCGGGCAATCTGAAGG



	TCTCC
Sec71-M717L-F	ATCATTctgCTGACCACGGATCTTCATT
Sec71-M717L-R	GGTCAGcagAATGATGCTGAATGCCAGC
Sec71-F713Y-F	GCTGGCAtacAGCATCATTATGCTGACCACG
Sec71-F713Y-R	GATGCTgtaTGCCAGCACGTAGACGG
pMT-ST::EGFP      pMT- ManII::EGFP	
Sac-ST	GATCTCGAGctcgccaccATGATTCACACCAACC TGAAGAAA
ST-Sal	GTACCGTCGACCCCTTGGTTTGCAATTTAAA GGAATC
Sac-ManII	GATCTCGAGctcgccaccATGAAGTTAAGTCGCC AGTTCACCG
ManII-B	GGTGGATCCAAACAGTCTCTGGGGTCAGCC T
pMT-GalT-EGFP-T2A- tdTomato-Rab11	
msK-GalT-F	ggggatctagatcggGGTACCaccATGAGGCTTCGGG AGC
Rab11-MT-Mlu	GATGGTGATGATGaccggtacgCGTACTGACAG CACTGTTTGCGCA
dT2A-EGFP	AGAGAACCCTGGCCCTggtGccaccATGGTGAG CAAGGGCGAG
dT2As	ggaGAAGGACGCGGCAGCCTACTGACTTGCG GAGATGTCTGAAGAGAACCCTGGCCCTggt
dT2Aa	GCCAGGGTTCTCTTCGACATCTCCGCAAGTC AGTAGGCTGCCGCGTCCTTcaccctcc

RNAi in S2 Cells	
garz-DRSC07193-F	CATATCGGGCGCACTATAATCTGCATTCCG
garz-DRSC07193-T7F	TAATACGACTCACTATAGGGGCATATCGGGCGC ACTATAATCTGCA
garz-DRSC07193-R	TTGCACAAACTTTGATTCTGCAGCA
garz-DRSC07193-T7F	TAATACGACTCACTATAGGGTTGCACAAACT TTGATTCTGCAGCA
garz-DRSC07193-T7R	TAATACGACTCACTATAGGGTTGCACAAACT TTGATTCTGCAGCA
Sec71-DRSC01893-F	AGTCCGATTTGCCCGAGGAGTACT
Sec71-DRSC01893-R	CATTATAAGCGTCTTGATGGTATCGATATTCT TGGC
Sec71-DRSC01893-T7F	TAATACGACTCACTATAGGGAGTCCGATTTG CCCGAG
Sec71-DRSC01893-T7R	TAATACGACTCACTATAGGGCATTATAAGCGT CTTGATGGTATCGAT
pMT-hyg-V5-iRFP713- Sec71	
GL3-Sec71	ggaggaggttctggtggtggtCACAACAACCTCCACAAA AACCAAGG
Sec71-MK-Sp	gatcttcatggtcgactagaATTATTTAACGCTCATTATA TTGAATACTGG
MK-MT-Ap	aggcttacctcgaaggcccagacagagtacttgcgctct
Asc-GL3	ggGGCGCGCCtGGAGGAGGAGGTTCTGGTGG TGGT
CRISPR-mediated genome-editing	
Sec71 BFA-resistant repairing template	
	GATCGGAAACTACATTGGCGAGAATGACGACCACTCCAAGGAAGTGAT GTGCGCTTACATCGATGCCTTTGACTTTCGCCAAATGGAGGTGGTGGCC



GCCTTGAGATTTCTTCTCGAGGGGTTCGCCTGCCAGGAGAAGCACAA  
AAAATCGATCGGCTGATGGAGAAGTTCGCCAGTAGATACTGCGAATGCA  
ATCCGAAGAAAtCAatTgTTtCAgtctGcTgAtACtGTgTAtGTtCTtGcTtTctATtA  
TcctgCTtACtACtGAcCTaCAcTtCtCAaGTgAAaCAtAAaATGACCAAGGAG  
CAGTACATTAATGAACCGCGGCATCAGCGACAGCAAGTCCGATTTGC  
CCGAGGAGTACTTGTCGTCCATCTACGACGAGATTTCTGAACACGAAAT  
TAAGATGAAGAACAACCTCCGGTATGCTTCAACAGGCGAAACCCACTGG  
AAAGCAGGCCTTCATAACGGAGAAACGCAGAAAGCTGTTGTGGA

Sec71 BFA-sensitive repairing template

GATCGGAAACTACATTGGCGAGAATGACGACCACTCCAAGGAAGTGAT  
GTGCGCTTACATCGATGCCTTTGACTTTCGCCAAATGGAGGTGGTGGCC  
GCCTTGAGATTTCTTCTCGAGGGGTTCGCCTGCCAGGAGAAGCACAA  
AAAATCGATCGGCTGATGGAGAAGTTCGCCAGTAGATACTGCGAATGCA  
ATCCGAAGAAAtCAatTgTTtCAgtctGcTgAtACtGTgTAtGTtCTtGcTtTaCtctATtA  
TcatgCTtACtACtGAcCTaCAcTtCtCAaGTgAAaCAtAAaATGACCAAGGAG  
CAGTACATTAATGAACCGCGGCATCAGCGACAGCAAGTCCGATTTGC  
CCGAGGAGTACTTGTCGTCCATCTACGACGAGATTTCTGAACACGAAAT  
TAAGATGAAGAACAACCTCCGGTATGCTTCAACAGGCGAAACCCACTGG  
AAAGCAGGCCTTCATAACGGAGAAACGCAGAAAGCTGTTGTGGA

crRNA for CRISPR-mediated genome-editing

Sec71-crRNA7

CUUUGGAAUAGCUGGUUCUUGUUUUAGAGCUAUGCU

(protospacer in DNA: CTTTGGAAATAGCTGGTTCTT)

Sec71-crRNA8

GGUCAAGCACAAGAUGACCAGUUUUAGAGCUAUGCU

(protospacer in DNA: GGTC AAGCACAAGATGACCA)

Table S2. DNA sequence of plasmids used in this study

pMK-V5::Sec71					
LOCUS	pMK_V5_Sec71	13255 bp	ds-DNA	circular	27-FEB-2020
COMMENT	pMK-V5-garz from 1 to 14245				
COMMENT	pMK33-CFH-BD from 1 to 8550				
COMMENT	>pMK33-CFH-BD, 8550 bases.				
COMMENT	ApEinfo:methylated:1				
FEATURES	Location/Qualifiers				
misc_feature	6493..6860				
	/label=MT-promoter				
	/ApEinfo_fwdcolor=cyan				
	/ApEinfo_revcolor=#00ff00				
	/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}				
	width 5 offset 0				
misc_feature	4326..5351				
	/label=Hyg				
	/ApEinfo_fwdcolor=#ffffcc				
	/ApEinfo_revcolor=#00ff00				
	/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}				
	width 5 offset 0				
misc_feature	6979..6984				
	/label=SpeI				
	/ApEinfo_fwdcolor=#ccff66				
	/ApEinfo_revcolor=#ffffcc				
	/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}				
	width 5 offset 0				
rep_origin	complement(777..1459)				
	/label=ColE1 origin				
	/ApEinfo_fwdcolor=gray50				
	/ApEinfo_revcolor=gray50				
	/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}				
	width 5 offset 0				
primer_bind	5645..5664				
	/label=pUAST-R				

```

/ApEinfo_fwdcolor=#0a00ff
/ApEinfo_revcolor=#0a00ff
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
CDS      complement(1557..2216)
/label=AmpR
/ApEinfo_fwdcolor=yellow
/ApEinfo_revcolor=yellow
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind  complement(2135..2159)
/label=Amp-GF
/ApEinfo_fwdcolor=#ff00bd
/ApEinfo_revcolor=#ff0003
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
polyA_signal  complement(6293..6484)
/label=SV40 late polyA
/ApEinfo_fwdcolor=pink
/ApEinfo_revcolor=pink
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind  2528..2545
/label=pQE60-F
/ApEinfo_fwdcolor=#ff00bd
/ApEinfo_revcolor=#ff0003
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind  1677..1700
/label=Amp-GR1
/ApEinfo_fwdcolor=#ff00bd
/ApEinfo_revcolor=#ff0003
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature  6937..6978
/label=V5
```



```

/ApEinfo_fwdcolor=#ffffcc
/ApEinfo_revcolor=#ffffcc
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature    6985..7008
                /label=Linker GL3
                /ApEinfo_fwdcolor=#ffcc66
                /ApEinfo_revcolor=#ffcc66
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind    complement(12340..12359)
                /label=Act5C-qR2
                /ApEinfo_fwdcolor=#ff00bd
                /ApEinfo_revcolor=#ff0003
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind    12144..12163
                /label=Act5C-qF1
                /ApEinfo_fwdcolor=#ff00bd
                /ApEinfo_revcolor=#ff0003
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind    complement(12351..12370)
                /label=Act5C-qR1
                /ApEinfo_fwdcolor=#ff00bd
                /ApEinfo_revcolor=#ff0003
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind    12078..12097
                /label=Act5C-qF2
                /ApEinfo_fwdcolor=#ff00bd
                /ApEinfo_revcolor=#ff0003
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind    complement(12047..12065)
                /label=Seq-MK-R
```

```
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature 12006..12027
    /label=New Feature
    /ApEinfo_fwdcolor=#ffffcc
    /ApEinfo_revcolor=#ffffcc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature 6928..6928
    /label=BamHI
    /ApEinfo_fwdcolor=#ffcc66
    /ApEinfo_revcolor=#ffcc66
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature 6896..6913
    /label=Metallothionein_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind 6836..6853
    /label=SEQ-MT-F2
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
CDS 7009..11964
    /label=Sec71
    /ApEinfo_fwdcolor=#99ccff
    /ApEinfo_revcolor=#cde7f7
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
CDS 9885..9885
    /label=Sec71-PA
```

```

    /ApEinfo_fwdcolor=#99ccff
    /ApEinfo_revcolor=#cde7f7
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
CDS      7972..7974
    /label=Sec71-PA(1)
    /ApEinfo_label=Sec71-PA
    /ApEinfo_fwdcolor=#99ccff
    /ApEinfo_revcolor=#cde7f7
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature 11562..11562
    /label=T>G silent
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature 11358..11358
    /label=T>C silent
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature 10504..10525
    /label=Chang-F1
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind 9765..9784
    /label=sec71-GF6
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind 7563..7582
```



```

        /label=sec71-GF3
        /ApEinfo_fwdcolor=#ff00bd
        /ApEinfo_revcolor=#ff0003
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature 9885..9885
        /label=A>C silent
        /ApEinfo_fwdcolor=cyan
        /ApEinfo_revcolor=#00ff00
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature 9624..9624
        /label=A>G in 40A
        /ApEinfo_fwdcolor=cyan
        /ApEinfo_revcolor=#00ff00
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
CDS 8064..8064
        /label=Sec71-PA(2)
        /ApEinfo_label=Sec71-PA
        /ApEinfo_fwdcolor=#99ccff
        /ApEinfo_revcolor=#cde7f7
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
CDS 8595..8595
        /label=Sec71-PA(3)
        /ApEinfo_label=Sec71-PA
        /ApEinfo_fwdcolor=#99ccff
        /ApEinfo_revcolor=#cde7f7
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
protein_bind 8728..9300
        /label=Sec7 domain
        /ApEinfo_fwdcolor=#0080ff
        /ApEinfo_revcolor=#0080ff
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}

```

```
width 5 offset 0
misc_feature 11442..11442
  /label=G>A silent
  /ApEinfo_fwdcolor=cyan
  /ApEinfo_revcolor=#00ff00
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 9145..9147
  /label=S191
  /ApEinfo_fwdcolor=#66ff66
  /ApEinfo_revcolor=#00ff00
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 11547..11547
  /label=C>T silent
  /ApEinfo_fwdcolor=cyan
  /ApEinfo_revcolor=#00ff00
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 11746..11746
  /label=T>C silent(1)
  /ApEinfo_label=T>C silent
  /ApEinfo_fwdcolor=cyan
  /ApEinfo_revcolor=#00ff00
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind 8545..8564
  /label=Sec71-GF5
  /ApEinfo_fwdcolor=#ff00bd
  /ApEinfo_revcolor=#ff0003
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind 9245..9268
  /label=Sec71-DRSC01893-F (8A10)
  /ApEinfo_fwdcolor=#ff00bd
  /ApEinfo_revcolor=#ff0003
```

```
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    complement(9721..9756)
    /label=Sec71-DRSC01893-R
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    11319..11319
    /label=T>C silent(2)
    /ApEinfo_label=T>C silent
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    11049..11068
    /label=sec71-GF8
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    10504..10525
    /label=sec71-GF7
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    complement(11219..11240)
    /label=Chang-R1
    /ApEinfo_fwdcolor=#fb53d0
    /ApEinfo_revcolor=#fc5a5d
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    7919..7938
    /label=sec71-GF4
    /ApEinfo_fwdcolor=#ff00bd
```



```
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind    complement(10557..10578)
    /label=Sec71-GR3
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature    9034..9036
    /label=E677 (E740 in garz)
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature    8595..8595
    /label=G>A silent(1)
    /ApEinfo_label=G>A silent
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature    8064..8064
    /label=C>T confirmed in 40A 2013
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature    9142..9144
    /label=F190 F190Y=BFA-hypersensitive
    /label=F713Y(BFA-sensitive)
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature    9552..9552
```

```
    /label=T>C in 40A
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature 9663..9663
    /label=A>C in 40A
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature 9714..9714
    /label=A>G 40A
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature 11331..11331
    /label=T>C in 40A(1)
    /ApEinfo_label=T>C in 40A
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind 11657..11674
    /label=Sec71-GF10
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind 7035..7052
    /label=GF9
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
```

```
primer_bind    9192..9210
               /label=Sec71-GF11
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
primer_bind    complement(9370..9393)
               /label=Sec71-GR4
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
primer_bind    8792..8815
               /label=Sec71-GF12
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
primer_bind    10132..10150
               /label=Sec71-GF13
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
misc_feature   9154..9156
               /label=M717(M194 in sec7 domain M>L = BFA-resistant)
               /label=M717L(BFA-resistant)
               /ApEinfo_fwdcolor=cyan
               /ApEinfo_revcolor=#00ff00
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
misc_feature   11965..11967
               /label=STOP
               /ApEinfo_fwdcolor=#66ccff
               /ApEinfo_revcolor=#00ff00
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
```



```
width 5 offset 0
primer_bind    8898..8920
               /label=Sec71-GF14
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    9068..9089
               /label=Sec71-GF15
               /ApEinfo_fwdcolor=#fb53d0
               /ApEinfo_revcolor=#fc5a5d
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature   complement(9094..9116)
               /label=Sec71-crRNA7(Protospacer)
               /ApEinfo_fwdcolor=#ccff66
               /ApEinfo_revcolor=#ffff9f
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature   9094..9096
               /label=PAM
               /ApEinfo_fwdcolor=#fc81f0
               /ApEinfo_revcolor=#fc81f0
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature   9183..9202
               /label=Sec71-crRNA8 Protospacer
               /ApEinfo_fwdcolor=#ccff66
               /ApEinfo_revcolor=#ffffcc
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature   9203..9205
               /label=PAM(1)
               /ApEinfo_label=PAM
               /ApEinfo_fwdcolor=#fc81f0
               /ApEinfo_revcolor=#fc81f0
```

```
      /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1} {} 0}
      width 5 offset 0
misc_feature 9823..9823
      /label=T>C Val>Ala in FRT40A
      /ApEinfo_fwdcolor=#ccff66
      /ApEinfo_revcolor=#00ff00
      /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1} {} 0}
      width 5 offset 0
ORIGIN
1 cccgcgttgc aggccatgct gtccaggcag gtagatgacg accatcaggg acagcttcaa
61 ggatcgctcg cggctcttac cagcctaact tcgatcattg gaccgctgat cgtcacggcg
121 atttatgccg cctcggcgag cacatggaac gggttggcat ggattgtagg cgccgcccta
181 taccttgtct gcctccccgc gttgcgtcgc ggtgcatgga gccgggccac ctcgacctga
241 atggaagccg gcggcacctc gctaacggat tcaccactcc aagaattgga gccaatcaat
301 tcttgcgag aactgtgaat gcgcaaacca acccttggca gaacatatcc atcgcgtccg
361 ccatctccag cagccgcacg cggcgcacat cgggcagcgt tgggtcctgg ccacgggtgc
421 gcatgatcgt gtcctgtcgc ttgaggacc ggctaggctg gcggggttgc cttactggtt
481 agcagaatga atcaccgata cgcgagcgaa cgtgaagcga ctgctgctgc aaaacgtctg
541 cgacctgagc aacaacatga atggtcttcg gtttccgtgt ttcgtaaagt ctgaaacgc
601 ggaagtcagc gctcttccgc ttctctgctc actgactcgc tgcgctcggg cgttcggctg
661 cggcgagcgg tatcagctca ctcaaaggcg gtaatacggg tatccacaga atcaggggat
721 aacgcaggaa agaacatgtg agcaaaaggc cagcaaaagg ccaggaaccg taaaaaggcc
781 gcgttgctgg cgtttttcca taggctccgc cccctgacg agcatcaca aaatcgacgc
841 tcaagtcaga ggtggcgaaa cccgacagga ctataaagat accaggcgtt tccccctgga
901 agctccctcg tgcgctctcc tgttccgacc ctgccgctta ccggatacct gtccgccttt
961 ctcccttcgg gaagcgtggc gctttctcat agctcacgct gtaggtatct cagttcggtg
1021 taggtcgttc gctccaagct gggctgtgtg cacgaacccc ccgttcagcc cgaccgctgc
1081 gccttatccg gtaactatcg tcttgagtc aaccggtaa gacacgactt atcgccactg
1141 gcagcagcca ctggtaacag gattagcaga gcgaggtatg taggcggtgc tacagagttc
1201 ttgaagtggg ggcctaacta cggctacact agaaggacag tatttggtat ctgcgctctg
1261 ctgaagccag ttaccttcgg aaaaagagtt ggtagctctt gatccggcaa acaaaccacc
1321 gctggtagcg gtggtttttt tgtttgcaag cagcagatta cgcgcagaaa aaaaggatct
1381 caagaagatc ctttgatctt ttctacgggg tctgacgctc agtggaacga aaactcacgt
1441 taagggattt tggatcatgag attatcaaaa aggatcttca ctagatcct tttaaattaa
1501 aatgaagtt ttaaatcaat ctaaagtata tatgagtaaa cttggtctga cagttaccaa
1561 tgcttaatca gtgaggcacc tatctcagcg atctgtctat ttcgttcatc catagttgcc
```

1621 tgactccccg tcgtgtagat aactacgata cgggagggct taccatctgg ccccagtgct  
1681 gcaatgatac cgcgagaccc acgtccaccg gctccagatt tatcagcaat aaaccagcca  
1741 gccggaaggg ccgagcgcag aagtggctct gcaactttat ccgcctccat ccagtctatt  
1801 aattgttgcc gggagctag agtaagtagt tcgccagtta atagtttgcg caacgttggt  
1861 gccattgctg caggcatcgt ggtgtcacgc tcgtcgtttg gtatggcttc attcagctcc  
1921 ggttcccaac gatcaaggcg agttacatga tccccatgt tgtgcaaaaa agcggttagc  
1981 tccttcggtc ctccgatcgt tgtcagaagt aagttggccg cagtgttatc actcatgggt  
2041 atggcagcac tgcataatc tcttactgtc atgccatccg taagatgctt ttctgtgact  
2101 ggtgagtact caaccaagtc attctgagaa tagtgtatgc ggcgaccgag ttgctcttg  
2161 ccggcgtcaa cacgggataa taccgcgcca catagcagaa ctttaaaagt gctcatcatt  
2221 ggaaaacgtt cttcggggcg aaaactctca aggatcttac cgctgttgag atccagttcg  
2281 atgtaacca ctcgtgcacc caactgatct tcagcatcct ttactttcac cagcgtttct  
2341 gggtgagcaa aaacaggaag gcaaatgcc gcaaaaaagg gaataagggc gacacggaaa  
2401 tgttgaatac tcatactctt ctttttcaa tattattgaa gcatttatca gggttattgt  
2461 ctcatgagcg gatacatatt tgaatgtatt tagaaaaata acaaatagg ggttcgcgc  
2521 acatttccc gaaaagtgcc acctgacgtc taagaaacca ttattatcat gacattaacc  
2581 tataaaaaata ggcgtatcac gaggcccttt cgtcttcaag aattcacatt tgtacgaatt  
2641 ttttttttat caaaagttcg agtttttcac caatttcctc atcaaccgag caaggcaaac  
2701 ggctttgaat aatattggtgt tatatataca tatatcaaat cgctgctgac tgcgtgattg  
2761 atggccccaa gattacatat tatcgaatca ggattcagaa ggagatcaat gtcaaatgcg  
2821 gacaggaaca tgaagacgc ctgttatgcy caattaaaaa tttgggttta attgctgtgg  
2881 aaactgttgt tggcggcatc ttaagttcct gtttaacaac atcaactact tatgtacgta  
2941 gaagcgttta agccatttg atacagatga gaactggctt ttgtgctaata cagtcaagat  
3001 gactccgatg atgatgactc attacctgac cagttttcgc tgctttcttt tcaacaacta  
3061 cttgtatatg tattgtatcc aatagcaata cattgaattt ccatggctca gtcacgtatt  
3121 atcatttaat tgacaccaag tcgtgttatt gttgagctat cgagttcagc tcaaacattt  
3181 cttattccca tgaataagcc ggcaaaaaata tgcaatctat gaaagttaat ataagcaaac  
3241 cttactttga ctcaatacca atgcactttg tgtcgatagg ttcacgcaat tgaggcgatt  
3301 attccgataa cccaagcgaat tgactgttcc cgtttcgatt ccaattgaaa tttggaaatg  
3361 tacaatagtt ttgctatatg ctgtcaagta cgctcttatc ttctctgggt tttcttcaga  
3421 gtttcgaaac gcttcttctt ttttttgttt tttttttttt ggaatctcgt attttggaaag  
3481 gggctcccct ctggaatttg ttacactgtc gttatcattg cgaacaagcg gcccgaaact  
3541 atcagcgact ttaacattta caatgcactt ttttacgacc aattaaatgt acattttcct  
3601 ttcttcgccc gttgataagc gaacgcgatg tggcgcaggc aatgtgttg tcttgcgaca  
3661 caaacgcaat caaatggat tcaatttcgc tttttccag tgaaacgaag aacgaaccga  
3721 ccatcatgat atgctcctct gcatgttgcy tattgaatca atgacaattt caattaagcc



3781 gcccgttcgt catgcgtttt cgtgcgcttc gaaatgctga taacgctgct gtcctccaac  
3841 tgctttgcat gtggacacaa ttccatttat ttaattcttt tatttgatc ggtaaatta  
3901 aaaagcgcct tgttacgcat ttaacgttgt ttccggtgcg tgggtggttc atgcttctgg  
3961 gaacggcaaa tgggtttagg attggaacc cctcatcadc tgttgaata tactattcaa  
4021 cctacaaaag taacgttaaa caacactact ttatatttga tatgaatggc cacacctttt  
4081 atgccataaa acatattgta agagaatacc actcttttta ttccttcttt ctttcttcta  
4141 cgttttttgc tgtaagtagg tcgtggtgct ggtggtgag ttgaaataac ttaaaatata  
4201 aatcataaaa ctcaaacata aacttgacta tttatttatt tattaagaaa ggaaatataa  
4261 attataaatt acaacagggt atggacctgc agccaagctt ggcgctcgtc cgggggcaat  
4321 gagatatgaa aaagcctgaa ctaccgcga cgtctgctga gaagtttctg atcgaaggt  
4381 tcgacagcgt ctccgacctg atgcagctct cggagggcga agaattctct gctttcagct  
4441 tcgatgtagg agggcgtgga tatgtcctgc gggtaaatag ctgcgccgat ggtttctaca  
4501 aagatcgtta tgtttatcgg cactttgcat cggccgcgct cccgattccg gaagtgcttg  
4561 acattgggga attcagcgag agcctgacct attgcatctc ccgccgtgca cagggtgtca  
4621 cgttgcaaga cctgcctgaa accgaactgc ccgctgttct gcagccggtc gcggaggcca  
4681 tggatgcatg cgctgcggcc gatcttagcc agacgagcgg gttcggccca ttcggaccgc  
4741 aaggaatcgg tcaatacact acatggcgtg atttcatatg cgcgattgct gatccccatg  
4801 tgtatcactg gcaaaactgt atggacgaca ccgtcagtcg gtccgctcgc caggctctcg  
4861 atgagctgat gctttgggcc gaggactgcc ccgaagtccg gcacctcgtg cacgcggatt  
4921 tcggctccaa caatgtcctg acggacaatg gccgcataac agcggtcatt gactggagcg  
4981 aggcgatggt cggggattcc caatacgagg tcgccaacat cttcttctgg aggccgtggt  
5041 tggcttgtat ggagcagcag acgcgctact tcgagcggag gcatccggag cttgcaggat  
5101 cgccgcggct ccgggcgtat atgctccgca ttggtcttga ccaactctat cagagcttgg  
5161 ttgacggcaa tttcgatgat gcagcttggg cgcagggctg atgcgacgca atcgtccgat  
5221 ccggagccgg gactgtcggg cgtacacaaa tcgcccgcag aagcgcggcc gtctggaccg  
5281 atggctgtgt agaagtactc gccgatagtg gaaaccgacg cccagcactc cgtccgaggg  
5341 caaaggaata gagtagatgc cgaccgaaca agagctgatt tcgagaacgc ctacagccagc  
5401 aactcgcgcg agcctagcaa ggcaaatgca agagaacggc cttacgcttg gtggcacagt  
5461 tctcgtccac agttcgttaa gctcgtcctg ctgggtcgcg ggagggccgg tcgcagtgat  
5521 tcaggccctt ctggattgtg ttggtcccca gggcacgatt gtcattccca cgcactcggg  
5581 tgatctgact gatcccgcag attggagatc gccgcccgtg cctgccgatt ggggtcagat  
5641 ctttgtgaag gaaccttact tctgtggtgt gacataattg gacaaactac ctacagagat  
5701 ttaaagctct aaggtaaata taaaattttt aagtgtataa tgtgttaaac tactgattct  
5761 aattgtttgt gtattttaga ttccaaccta tggaaactgat gaatgggagc agtgggtgaa  
5821 tgcctttaat gaggaaaacc tgttttgctc agaagaaatg ccatctagtg atgatgaggc  
5881 tactgctgac tctcaacatt ctactcctcc aaaaaagaag agaaaggtag aagaccccaa

5941 ggactttcct tcagaattgc taagtttttt gagtcatgct gtgttttagta atagaactct  
6001 tgcttgcttt gctatttaca ccacaaagga aaaagctgca ctgctataca agaaaattat  
6061 ggaaaaatat tctgtaacct ttataagtag gcataacagt tataatcata acatactgtt  
6121 ttttcttact ccacacaggc atagagtgtc tgctattaat aactatgctc aaaaattgtg  
6181 taccttttagc tttttaattt gtaaaggggt taataaggaa tatttgatgt atagtgcctt  
6241 gactagagat cataatcagc cataccacat ttgtagaggt tttacttgct ttaaaaaacc  
6301 tcccacacct cccctgaac ctgaaacata aatgaatgc aattgttgtt gttaacttgt  
6361 ttattgcagc ttataatggt tacaataaa gcaatagcat cacaaattc acaataaag  
6421 catttttttc actgcattct agttgtggtt tgtccaaact catcaatgta tcttatcatg  
6481 tctggatcaa ttcgttgag gacaggatgt ggtgcccgat gtgactagct ctttgctgca  
6541 ggccgtccta tcctctggtt ccgataagag acccagaact ccggccccc accgccacc  
6601 gccaccccca tacatatgtg gtacgcaagt aagagtgcct gcgcatgccc catgtgcccc  
6661 accaagagct ttgcatccca tacaagtccc caaagtggag aaccgaacca attcttcgcg  
6721 ggacagaaca aagcttctgc acacgtctcc actcgaattt ggagccggcc ggcgtgtgca  
6781 aaagaggtga atcgaacgaa agaccctgtt gtaaagccgc gtttccaaa tgtataaac  
6841 cgagagcatc tggccaatgt gcatcagttg tggtcagcag caaatcaag tgaatcatct  
6901 cagtgcaact aaagggggga tctcagggcc accATGGGTA AGCCTATCCC TAACCCTCTC  
6961 CTCGGTCTCG ATTCTACGAC TAGTGGAGGA GGAGGTTCTG GTGGTGGTCA CAACAACCTCC  
7021 ACAAAAACCA AGGAAATGTT CATCGTGCGT GCTCTAGAAA AGATCCTTGC CGATAAGGAC  
7081 ATACGGCGCT CCCATCACTC GCAGCTGAAG AAGTCCTGCG ATTCGGCGCT GGAGCAGATT  
7141 AAGGCGGAGC TAATCAGTGC CGGCCAGATC GCAGAGGGCA ATGAGCTGCC CTGTGCCGCA  
7201 CTCCCCTGTC CCAAGAATGA TGCAGCGAGC ATCATAAATG CGGAGACCTA CTTTCTCCCC  
7261 TTCGAGCTTG CCTGCAAGAG CCGCTCGCCC AGGATCGTGG TCACCGCACT GACTGCCTG  
7321 CAGAACTCA TTGCCTATGG CCATTTGACA GGATCCATTC AGGACTCGGC CAATCCGGGT  
7381 CACCTGCTCA TCGACCGTAT CGTTGTGACC ATATATGGCT GCTTCAGTGG TCCCAGACG  
7441 GACGAGGCCG TCCAACCTGCA GATAATAAAG GCTCTGCTCA CGGTGGTAC CTCGCAGCAT  
7501 GTGGAATCC ATGAATTCAC ACTGCTGCAA GCTGTGCGCA CCTGCTACGA CATCTATTG  
7561 TCCAGCAAGA ACCTGGTCAA TCAGACCACA GCACGCGCTA CGCTCACCCA AATGTTGAAC  
7621 GTGATATTTG CCCGCATGGA GAATCAAGTG TACGAGCTAC CACCTCCAA TTCCAATCCC  
7681 ACCAACGGCA GCATCCACTC GGAGGATTGC AATGGCTCGG GAGAGGAGTC GCTGCGGGAT  
7741 TCCGACGAAG TAATTGCCTC GGAAGTCTG GCGGAGATCA TATCAGCTGC CTACAATGAG  
7801 GCGATGAAGG ATCAGGAATC GGTCGGTGAG CCAGAGCCAA CACTTAATGG AAACGACTAC  
7861 TCCTCGCACT CGGATCACGA CAGTGTGGAG CTGCACAGCG AAAACGATGC GGTGTAAACG  
7921 GCTAAGTTTA CGCACATCCT GCAGAAAGAT GCTTTTCTCG TGTTCCGGGC ACTGTGCAAG  
7981 CTATCGATGA AGCCTTTGCC GGATGGACAT CCAGATCCGA AATCGCACGA GCTGCGTTCC  
8041 AAGGTGCTGT CATTGCATCT GCTGCTGCTC ATCCTCCAGA ATGCCGGGCC CGTCTTCCGC

8101 TCCAACGAGA TGTTATCAT GGCCATTAAG CAGTACCTGT GCGTGGCCTT GTCAAACAAC  
8161 GGAGTCAGTC TGGTGCCGA GGTCTTCGAG CTGTGCTTT CAATCTTCGT TGCCCTACTC  
8221 TCGAACTTCA AGGTGCATCT TAAGCGGCAG ATAGAGGTGT TCTTCAAGGA AATCTTCCTA  
8281 AACATTCTTG AGGCGAACTC AAGCAGCTTC GAGCACAAAT GGATGGTAAT CCAAGCGCTG  
8341 ACACGTATTT GTGCTGACGC CCAGTCCGTG GTGGATATCT ATGTTAATTA CGATTGCGAC  
8401 TTTTCGGCTG CAAACCTTTT TGAGAGACTG GTCAACGATC TTTTCGAAAAT TGCCCAGGGT  
8461 CGTCAGGCTC TCGAACTGGG CGCCAATCCG ATGCAAGAGA AATCGATGCG CATTGCGGCG  
8521 CTGGAGTGTC TTGTCTCCAT TCTTAAGTGC ATGGTAGAGT GGAGTAAGGA CTTGTATGTT  
8581 AATCCAAACA TGCCGGTTCC ACCTATGCAA GTCCAATCGC CGACAAGCAC TGAGCAGGAT  
8641 CAGGCGGACA CAACTATCCA AACGATGCAC AGTGGTTCGA GTCATAGTTT GAACTCCAAT  
8701 CAGGAGCAAC TACAGGATCT TCCCGAGGCA TTGGAGGAGC GCAAGATGCG CAAGGAAGTG  
8761 ATGGAAACAG GCATTGAGTT ATTCAATCGT AAGCCTCAGA AAGGAGTGCA ATTCCTGCAG  
8821 GAGAAGCAGT TGCTGGGTGC CACATGCGGG GACATTGCGC GCTGGCTGCA CGAGGACGAA  
8881 CGACTGGACA AGACAGTGAT CGGAAACTAC ATTGGCGAGA ATGACGACCA CTCCAAGGAA  
8941 GTGATGTGCG CTTACATCGA TGCCTTTGAC TTTTCGCCAAA TGGAGGTGGT GGCCGCCTTG  
9001 AGATTTCTTC TCGAGGGGTT CCGCTGCCA GGAGAAGCAC AAAAAATCGA TCGGCTGATG  
9061 GAGAAGTTCG CCAGTAGATA CTGCGAATGC AATCCGAAGA ACCAGCTATT CCAAAGCGCA  
9121 GACACCGTCT ACGTGCTGGC ATTCAGCATC ATTATGCTGA CCACGGATCT TCATTGCGCC  
9181 CAGGTCAAGC ACAAGATGAC CAAGGAGCAG TACATTAATA TGAACCGCGG CATCAGCGAC  
9241 AGCAAGTCCG ATTTGCCCGA GGAGTACTTG TCGTCCATCT ACGACGAGAT TTCTGAACAC  
9301 GAAATTAAGA TGAAGAACAA CTCCGGTATG CTTCAACAGG CGAAACCCAC TGGAAAGCAG  
9361 GCCTTCATAA CGGAGAAACG CAGAAAGCTG TTGTGGAACA TGGAGATGGA GGTCATCTCG  
9421 CTGACGGCCA CCAATCTAAT GCAGTCAGTT TCGCACGTCA AGTCACCCTT CACCTCAGCG  
9481 AAACACTTGG AGCATGTCCG GCCCATGTTC AAAATGGCTT GGACACCATT TCTGGCCGCT  
9541 TTCTCTGTGG GTCTCCAGGA CTGCGACGAT CCTGAGATTG CTACACTCTG CTTGGATGGT  
9601 ATACGTTGTG CTATTCGAAT CGCATGCATC TTCCACATGT CCCTGGAGCG AGATGCCTAT  
9661 GTACAAGCCC TGGCCAGGTT TACTCTCCTG AATGCTAACT CGCCCATCAA CGAAATGAAG  
9721 GCCAAGAATA TCGATACCAT CAAGACGCTT ATAATGGTAG CCCACACGGA TGGCAATTAT  
9781 CTGGGCAGCA GCTGGCTGGA TATAGTGAAG TGCATTAGCC AGTTGGAGCT GGCCCAACTG  
9841 ATCGGCACTG GGGTGC GGCC CCAGTTTCTT TCTGGAGCGC AGACAACGCT CAAGGACTCG  
9901 CTTAATCCCA GCGTGAAAGA ACACATCGGC GAGACGAGCA GCCAGAGCGT GGTGGTCGCA  
9961 GTCGATCGTA TTTTCACCGG CTCAATGCGA CTGGATGGCG ATGCTATCGT GGACTTCGTG  
10021 AAGGCCCTGT GCCAGGTGTC TGTGGATGAG CTTCAGCAGC AGCAACCGAG GATGTTCTCC  
10081 TTGCAAAAGA TAGTGAAAT TAGTTACTAC AACATGGAGC GTATTCGTCT GCAGTGGTCA  
10141 CGCATTTGGC AAGTTTTGGG TGAGCACTTT AACCGGTCG GATGCAATAG CAACGAGGAG  
10201 ATCTCATTTT TCGCCCTGGA CTCACTGCGT CAGTTGTCGA TGAAGTTCAT GGAGAAGGGC

10261 GAGTTCAGTA ATTTCCGCTT CCAGAAGGAT TTCCTGCGTC CCTTTGAGCA TATCATGAAG  
10321 AAAAACGCAT CGCCGGCAAT ACGAGATATG GTGGTGCCT GCATTGCCA GATGGTAAAC  
10381 TCACAGGCGC ATAACATCCG TTCCGGCTGG AAGAATATCT TTAGCATTTC CCACCTGGCA  
10441 GCGGGAGACA ACGAAGAGCC AATTGTGGAG CTGGCCTTCC AAACCACGGG CAAAATCATC  
10501 GGTGATCTGT ACAAGCGTCA GTTCGCCATT ATGGTGGACT CGTTCAGGA TGCGGTCAAG  
10561 TGCCTGTCAG AGTTCGCCAC CGCCAGATTC CCCGATACCA GCATGGAAGC CATACTGTCTG  
10621 GTCCGTACCT GCGCGCAGTG CGTCCACGAG GCACCACAAC TGTTTGCGGA GCATGCCGGC  
10681 ATGGAGAACG ACGCCTCGGT GGCCGAGGAG GATCGAGTCT GGGTGCCTGG CTGGTTTCCG  
10741 ATGCTATTCT CGCTTTCCTG CGTGGTCAAT CGCTGCAAAT TGGATGTGCG TACTCGCGCC  
10801 TTAACCGTGC TTTTTGAGAT TGTGAAGACG TATGGTGAGA GCTTCAAGCC CCATTGGTGG  
10861 AAGGATCTCT TCAATGTGAT CTTCCGTATC TTCGACAACA TGAAATTGCC GGAGCACGTC  
10921 ACCGAGAAGT CCGAATGGAT GACGACCACA TGCAACCACG CCTTGTACGC TATTATTGAT  
10981 GTCTTCACGC AGTATTTGCA TGTTCTTGGT CATCTGCTGC TGGAGGAGCT CTTGCCCCAG  
11041 CTGCATTGGT GTGTTTCAGCA GAGTAACGAG CAGTTGGCGC GATCTGGCAC CAATTGCCTG  
11101 GAGAACCTCG TCATTTGAA TGGATTCAAG TTCAACGAGT CCACCTGGGA CAAGACGTGC  
11161 CAGTGCATCC TGGACATCTT CAACGCCACT TTGCCGAGG ATCTCCTCAG TTGGCGGCCG  
11221 AAAGCACATT CCAGTAACAA TATACCCAG GAGACAACC ACTTTGAGGC GCTGCATATC  
11281 CGTGCGTAG TCCAGCTGGA ACTGATACAG ACCATGGATA ACATTGTCTT TTTCCCGGCC  
11341 ACGTCGCGCA AGGAGGATGC CGAAACGCTG GCCCAGGCGG CGGCAGACTT AACAGGCGGC  
11401 AGGAGCGGTT CGCAGTCGCA GCTGCTGGAG TGCCAGCGGG AGGAGCAGGG AATGTACGGC  
11461 TATCTGAGAA CCCGCCAGCT GCTCACCTG GCCGACTGTC TGATGCAGTC GCACCGTTTT  
11521 GCCAAGCGCT TCAACGCCA TCACGACCAA CGCAGCCTGC TTTGGCGGG GGGATTCAAG  
11581 GGATCTGTTA AACCGAATCT GCTGAAGCAG GAGACCTCGT CGCTGGCCTG CGTCTGCGC  
11641 ATTTTCTTCA AGATGTACGG CGACGAGAAT AGACGCAGCG ATTTGGCCCG CATCGAGCAG  
11701 GAACTGGTGC AGGTCTGCAA GGAGGCACTG GGCTACTATT TGAGTTTGCA GAGCGAGGCA  
11761 CACCGAGATG CGTGGACATC GCTGCTGCTG CTCATCCTGA CGCGCCTGCT CAAGATGTCC  
11821 GATGCCAGGT TCGCCACCCA CGTTTCCAAC TACTACAGCC TGCTGTGCGA GATGATGTGC  
11881 TTCGACCTCA AGCCCGAAGT GAGAAGTGTG CTTAGGCGTG TGTTTCATGCG CATCGGTCCA  
11941 GTATTCAATA TAATGAGCGT TAAATAAttc tagtcgacca tgaagatcaa gatcattgcc  
12001 ccgccagagc gcaagtactc tgtctggatc ggtggctcca tcctggcttc gctgtccacc  
12061 ttccagcaga tgtggatctc caagcaggag tacgacgagt ccggcccctc catttgtcac  
12121 cgcaagtgtc tctaagaagg atcgcttgtc tgggcaagag gatcaggatc gggatggtct  
12181 tgattctgct ggaggaggag gaggagaagt cgaggaagca gcagcgaag tgcaagtgcg  
12241 agtggaggaa gtttggagtg cagcacaaca aatcaaca caacaccaac tacaagatga  
12301 aaagagcgga accacctgca caccatcatc actatcatca tcgttttggg cgcatgttgt  
12361 gtggttcag cgtattaata taattaatth attccacatg agatatgata tgatatacta



<pre> 12421 tgtatTTTTT gTTTTTTTT tatttGtaaa ctttaatat aacaagaact acaaaaaatg 12481 aaaatgagcg aaaatgcata ttctgccatt ccacacacac accaacaaca cccaacacac 12541 gcacaccac aagcttacac acacacattc gcggcatgac aaggacatca agataaagaa 12601 gaacttaaag aagatatttc ccaagcgca aaaagaacac acacacattg caaaacacaa 12661 acaacacact agcgTTTTgt acaattcgtc agcaacctta tgtattattt tttattatg 12721 atgtaattat aaacaaagtg aaacaaaaat atgaaaacaa aaagggaaat caaatctgtc 12781 ttctctttct cccgctctcc tcgctctctg ctgctaacct cgcctctcc tctctcatct 12841 tttgtctgt ctctcttcca catttttgc ggccggcaaa ataataacc acacacactc 12901 acacttggt gcagtttcgc gtgcgatatt cacacacatt caagcataca agcatacata 12961 catatgtatt tttttttat ttgtacactt ttctaattgc atgcgatcg attgataagt 13021 ttacgcctga aatgttaat taaaatgtga aatgcaact gaaaaactga tgaaatgaaa 13081 caacaacaag cgaacaattt gctacatgtg tattgtctaa caaccgttac tgcaacggtt 13141 gcttcgaaa aggggtgaaga ggaagagggc acgaggtcga ctagagcggc cgccgacgcg 13201 aggctggatg gccttccca ttatgattct tctcgcttcc ggccgcatcg ggatg // </pre>					
pMK-V5::garz					
LOCUS	pMK_V5_garz	14245 bp	ds-DNA	circular	27-FEB-2020
COMMENT	pMK33-CFH-BD from 1 to 8550				
COMMENT	>pMK33-CFH-BD, 8550 bases.				
COMMENT	ApEinfo:methylated:1				
FEATURES	Location/Qualifiers				
CDS	7009..8862				
	/label=garz-PB				
	/ApEinfo_fwdcolor=#99ccff				
	/ApEinfo_revcolor=#cde7f7				
	/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}				
	width 5 offset 0				
CDS	8908..9273				
	/label=New Feature				
	/ApEinfo_fwdcolor=#99ccff				
	/ApEinfo_revcolor=#99ccff				
	/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}				
	width 5 offset 0				
misc_feature	6493..6860				
	/label=MT-promoter				

```
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    4326..5351
    /label=Hyg
    /ApEinfo_fwdcolor=#ffffcc
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    6979..6984
    /label=SpeI
    /ApEinfo_fwdcolor=#ccff66
    /ApEinfo_revcolor=#ffffcc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    6928..6928
    /label=BamHI
    /ApEinfo_fwdcolor=#ffcc66
    /ApEinfo_revcolor=#ffcc66
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    11071..11088
    /label=garz-GF9
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    6896..6913
    /label=Metallothionein_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
CDS            11089..12954
    /label=garz-PB(1)
```

```

    /ApEinfo_label=garz-PB
    /ApEinfo_fwdcolor=#99ccff
    /ApEinfo_revcolor=#cde7f7
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    10513..10532
    /label=garz-GF8
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
rep_origin    complement(777..1459)
    /label=ColE1 origin
    /ApEinfo_fwdcolor=gray50
    /ApEinfo_revcolor=gray50
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature    9325..9327
    /label=190Y
    /ApEinfo_fwdcolor=#ccff66
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
CDS    complement(1557..2216)
    /label=AmpR
    /ApEinfo_fwdcolor=yellow
    /ApEinfo_revcolor=yellow
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    8863..8883
    /label=garz-GF5
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
CDS    7555..7581
```

```
    /label=New Feature(1)
    /ApEinfo_label=New Feature
    /ApEinfo_fwdcolor=#99ccff
    /ApEinfo_revcolor=#99ccff
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    9414..9431
    /label=garz-GF6
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
polyA_signal    complement(6293..6484)
    /label=SV40 late polyA
    /ApEinfo_fwdcolor=pink
    /ApEinfo_revcolor=pink
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    7555..7581
    /label=JF01603-F
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature    9490..9709
    /label=Phosphatidylinositol-phosphate binding
    /ApEinfo_fwdcolor=#ff81f0
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    5645..5664
    /label=pUAST-R
    /ApEinfo_fwdcolor=#0a00ff
    /ApEinfo_revcolor=#0a00ff
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
```



```
primer_bind    9916..9935
               /label=garz-GF7
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
primer_bind    13134..13153
               /label=Act5C-qF1
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
misc_feature   10941..10977
               /label=Phosphatidylinositol-phosphate binding(1)
               /ApEinfo_label=Phosphatidylinositol-phosphate binding
               /ApEinfo_fwdcolor=#ff81f0
               /ApEinfo_revcolor=#00ff00
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
primer_bind    complement(13341..13360)
               /label=Act5C-qR1
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
CDS            9710..10940
               /label=Phosphatidylinositol-phosphate binding(2)
               /ApEinfo_label=Phosphatidylinositol-phosphate binding
               /ApEinfo_fwdcolor=#ff81f0
               /ApEinfo_revcolor=#99ccff
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
primer_bind    13068..13087
               /label=Act5C-qF2
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
```

		/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
		width 5 offset 0
primer_bind	7115..7134	
		/label=garz-GF3
		/ApEinfo_fwdcolor=#ff00bd
		/ApEinfo_revcolor=#ff0003
		/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
		width 5 offset 0
CDS	10978..11070	
		/label=garz-PB(2)
		/ApEinfo_label=garz-PB
		/ApEinfo_fwdcolor=#99ccff
		/ApEinfo_revcolor=#cde7f7
		/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
		width 5 offset 0
primer_bind	complement(13330..13349)	
		/label=Act5C-qR2
		/ApEinfo_fwdcolor=#ff00bd
		/ApEinfo_revcolor=#ff0003
		/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
		width 5 offset 0
CDS	8863..8883	
		/label=New Feature(2)
		/ApEinfo_label=New Feature
		/ApEinfo_fwdcolor=#99ccff
		/ApEinfo_revcolor=#99ccff
		/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
		width 5 offset 0
CDS	11071..11088	
		/label=New Feature(3)
		/ApEinfo_label=New Feature
		/ApEinfo_fwdcolor=#99ccff
		/ApEinfo_revcolor=#99ccff
		/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
		width 5 offset 0
CDS	10941..10977	

```
    /label=New Feature(4)
    /ApEinfo_label=New Feature
    /ApEinfo_fwdcolor=#99ccff
    /ApEinfo_revcolor=#99ccff
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    2528..2545
    /label=pQE60-F
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    complement(2135..2159)
    /label=Amp-GF
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature   10364..10365
    /label=MB05159
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    11710..11731
    /label=garz-GF10
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    1677..1700
    /label=Amp-GR1
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
```

```
misc_feature    9274..9482
                /label=Sec7 domain
                /ApEinfo_fwdcolor=#0080ff
                /ApEinfo_revcolor=#2098da
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
                width 5 offset 0
primer_bind     6836..6853
                /label=SEQ-MT-F2
                /ApEinfo_fwdcolor=#ff00bd
                /ApEinfo_revcolor=#ff0003
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
                width 5 offset 0
misc_feature    9328..9330
                /label=191A
                /ApEinfo_fwdcolor=cyan
                /ApEinfo_revcolor=#00ff00
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
                width 5 offset 0
primer_bind     complement(13037..13055)
                /label=Seq-MK-R
                /ApEinfo_fwdcolor=#ff00bd
                /ApEinfo_revcolor=#ff0003
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
                width 5 offset 0
misc_feature    9483..9489
                /label=Sec7 domain(1)
                /ApEinfo_label=Sec7 domain
                /ApEinfo_fwdcolor=#0080ff
                /ApEinfo_revcolor=#00ff00
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
                width 5 offset 0
CDS             8908..9273
                /label=Sec7 domain
                EQLAKVKQKKRLLSQGTERFNQRPEKGIQYLQEHGILNAELD
                /ApEinfo_fwdcolor=#0080ff
                /ApEinfo_revcolor=#99ccff
```



```

    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    6985..7008
    /label=Linker GL3
    /ApEinfo_fwdcolor=#ffcc66
    /ApEinfo_revcolor=#ffcc66
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
CDS            8884..8907
    /label=garz-PB(3)
    /ApEinfo_label=garz-PB
    /ApEinfo_fwdcolor=#99ccff
    /ApEinfo_revcolor=#cde7f7
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    7726..7744
    /label=garz-GF12
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    12328..12346
    /label=garz-GF11
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
CDS            9490..9709
    /label=New Feature(5)
    /ApEinfo_label=New Feature
    /ApEinfo_fwdcolor=#99ccff
    /ApEinfo_revcolor=#99ccff
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    9729..9757
    /label=garz-7193-F
```

```
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature    6937..6978
    /label=V5
    /ApEinfo_fwdcolor=#ffffcc
    /ApEinfo_revcolor=#ffffcc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
CDS
    9483..9489
    /label=New Feature(6)
    /ApEinfo_label=New Feature
    /ApEinfo_fwdcolor=#99ccff
    /ApEinfo_revcolor=#99ccff
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    complement(10211..10236)
    /label=garz-7193-R
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature    9223..9225
    /label=E740K
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature    8000..8022
    /label=Rab1 binding
    /ApEinfo_fwdcolor=#66ccff
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    complement(8000..8022)
```

```
        /label=garz-JF01603-R
        /ApEinfo_fwdcolor=#ff00bd
        /ApEinfo_revcolor=#ff0003
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
misc_feature    7923..7923
        /label=New Feature(7)
        /ApEinfo_label=New Feature
        /ApEinfo_fwdcolor=#ffffcc
        /ApEinfo_revcolor=#00ff00
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
misc_feature    7931..7931
        /label=New Feature(8)
        /ApEinfo_label=New Feature
        /ApEinfo_fwdcolor=#ffffcc
        /ApEinfo_revcolor=#00ff00
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
primer_bind     8238..8256
        /label=garz-GF4
        /ApEinfo_fwdcolor=#ff00bd
        /ApEinfo_revcolor=#ff0003
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
```

ORIGIN

```
1 cccgcgtgc aggccatgct gtccaggcag gtagatgacg accatcaggg acagcttcaa
61 ggatcgctcg cggctcttac cagcctaact tcgatcattg gaccgctgat cgtcacggcg
121 atttatgccg cctcggcgag cacatggaac gggttggcat ggattgtagg cgccgcccta
181 taccttgtct gcctccccgc gttgcgtcgc ggtgcatgga gccgggccac ctcgacctga
241 atggaagccg gcggcacctc gctaacggat tcaccactcc aagaattgga gccaatcaat
301 tcttgcgag aactgtgaat gcgcaaacca acccttgca gaacatatcc atcgcgtccg
361 ccatctccag cagccgcacg cggcgcattc cgggcagcgt tgggtcctgg ccacgggtgc
421 gcatgatcgt gtcctgtcg ttgaggacc ggctaggctg gcgggggtgc cttactggtt
481 agcagaatga atcaccgata cgcgagcga cgtgaagcga ctgctgctgc aaaacgtctg
541 cgacctgagc aacaacatga atggctctcg gtttccgtgt ttcgtaaagt ctggaacgc
```

601 ggaagtcagc gctcttccgc ttcctcgcctc actgactcgc tgcgctcggc cgttcggctg  
661 cggcgagcgg taccagctca ctcaaaggcg gtaatacggc tatccacaga atcaggggat  
721 aacgcaggaa agaacatgtg agcaaaaggc cagcaaaagg ccaggaaccg taaaaaggcc  
781 gcgttgctgg cgtttttcca taggctccgc cccctgacg agcatcaca aaatcgacgc  
841 tcaagtcaga ggtggcgaaa cccgacagga ctataaagat accaggcgtt tccccctgga  
901 agctccctcg tgcgctctcc tgttccgacc ctgccgctta ccggatacct gtccgccttt  
961 ctcccttcgg gaagcgtggc gctttctcat agctcacgct gtaggtatct cagttcggtg  
1021 taggtcgttc gctccaagct gggctgtgtg cacgaacccc ccgttcagcc cgaccgctgc  
1081 gccttatccg gtaactatcg tcttgagtc aaccggtaa gacacgactt atcgccactg  
1141 gcagcagcca ctggtaacag gattagcaga gcgaggtatg taggcggtgc tacagagttc  
1201 ttgaagtggg ggcctaacta cggctacact agaaggacag tatttggtat ctgcgctctg  
1261 ctgaagccag ttaccttcgg aaaaagagtt ggtagctctt gatccggcaa acaaacacc  
1321 gctggtagcg gtggtttttt tgtttgcaag cagcagatta cgcgcagaaa aaaaggatct  
1381 caagaagatc ctttgatctt ttctacgggg tctgacgctc agtggaacga aaactcacgt  
1441 taagggatth ttggtcatgag attatcaaaa aggatcttca cctagatcct tttaaattaa  
1501 aatgaagtt ttaaatcaat ctaaagtata tatgagtaaa cttggtctga cagttaccaa  
1561 tgcttaatca gtgaggcacc tatctcagcg atctgtctat ttcgttcacc catagttgcc  
1621 tgactccccg tcgtgtagat aactacgata cgggagggct taccatctgg cccagtgct  
1681 gcaatgatac cgcgagacc acgctcaccg gctccagatt tatcagcaat aaaccagcca  
1741 gccggaaggg ccgagcgcag aagtggctct gcaactttat ccgctccat ccagtctatt  
1801 aattggtgcc ggaagctag agtaagtagt tcgccagtta atagtttgcg caacgttggt  
1861 gccattgctg caggcatcgt ggtgtcacgc tcgtcgtttg gtatggcttc attcagctcc  
1921 ggttcccaac gatcaaggcg agttacatga tccccatgt tgtgcaaaaa agcggtagc  
1981 tccttcggct cccgatcgt tgtcagaagt aagttggccg cagtgttacc actcatggtt  
2041 atggcagcac tgcataattc tcttactgtc atgccatccg taagatgctt ttctgtgact  
2101 ggtgagtact caaccaagtc attctgagaa tagtgatgc ggcgaccgag ttgctctg  
2161 ccggcgtcaa cacgggataa taccgcgcca catagcagaa ctttaaaagt gctcatcatt  
2221 ggaaaacggt cttcggggcg aaaactctca aggatcttac cgctgttgag atccagttcg  
2281 atgtaacca ctcgtgcacc caactgatct tcagcatctt ttactttcac cagcgtttct  
2341 gggtagcaaa aaacaggaag gcaaaatgcc gcaaaaaagg gaataagggc gacacggaaa  
2401 tgttgaatac tcatactctt ctttttcaa tattattgaa gcatttatca gggttattgt  
2461 ctcatgagcg gatacatatt tgaatgtatt tagaaaaata acaaaatagg ggttccgcgc  
2521 acatttcccc gaaaagtgcc acctgacgct taagaaacca ttattatcat gacattaacc  
2581 tataaaaaata ggcgtatcac gaggcccttt cgtcttcaag aattcacatt tgtacgaatt  
2641 ttttttttat caaaagttcg agtttttcac caatttcctc atcaaccgag caaggcaaac  
2701 ggctttgaat aatattggtg tatatataca tatatcaaat cgctgctgac tgcgtgattg

2761 atggccccaa gattacatat tatcgaatca ggattcagaa ggagatcaat gtcaaatgcg  
2821 gacaggaaca tgaagagcgc ctgttatgcg caattaaaaa tttgggttta attgctgtgg  
2881 aaactgtttg tggcggcatc ttaagttcct gtttaacaac atcaactact tatgtacgta  
2941 gaagcgttta agccatttgc atacagatga gaactggcct ttgtgcta atcagtcagat  
3001 gactccgatg atgatgactc attacctgac cagttttcgc tgctttcttt tcaacaacta  
3061 cttgtatatg tattgtatcc aatagcaata cattgaattt ccatggctca gtcacgtatt  
3121 atcattta at tgacaccaag tcgtgttatt gttgagctat cgagttcagc tcaaacattt  
3181 cttattccca tgaataagcc ggcaaaaata tgcaatctat gaaagtta ataaagcaaac  
3241 cttactttga ctcaatacca atgcactttg tgtc gatagg ttcacgcaat tgaggcgatt  
3301 attccgataa cccaagcgat tgactgttcc cgtttcgatt ccaattgaaa tttggaaatg  
3361 tacaatagtt ttgctatatg ctgtcaagta cgctcttacc ttctctgggt tttcttcaga  
3421 gtttcgaaac gcttcttctt ttttttgttt tttttttttt ggaatctcgt attttggaa  
3481 gggctcccct ctggaatttg ttacactgtc gttatcattg cgaacaagcg gcccgaaagt  
3541 atcagcgact ttaacattta caatgcactt ttttacgacc aattaaatgt acattttcct  
3601 ttcttcgccc gttgataagc gaacgcgatg tggcgcaggc aatgtgttg tcttgcgaca  
3661 caaacgcaat caaaatggat tcaatttcgc tttttccag tgaacgaag aacgaaccga  
3721 ccatcatgat atgctcctct gcatgttgcg tattgaatca atgacaattt caattaagcc  
3781 gcccgttcgt catgctgttt cggtgccttc gaaatgctga taacgctgct gtcctcaac  
3841 tgctttgcat gtggacacaa ttccatttat ttaattcttt tatttggatc ggttaaatta  
3901 aaaagcgcct tgttacgcat ttaacgttgt ttccggtgcg tgggtggttc atgcttctgg  
3961 gaacggcaaa tgggttttagg attggaacc cctcatcacc tggttggaata tactattcaa  
4021 cctacaaaag taacgttaaa caacactact ttatatttga tatgaatggc cacacctttt  
4081 atgccataaa acatattgta agagaatacc actcttttta ttcttcttt ccttcttga  
4141 cgttttttg tgaagtagg tcgtggtgct ggtgttgagc ttgaaataac ttaaaatata  
4201 aatcataaaa ctcaaacata aacttgacta tttatttatt tattaagaaa ggaatataa  
4261 attataaatt acaacaggtt atggacctgc agccaagctt ggcgctcgtc cgggggcaat  
4321 gagatATGAA AAAGCCTGAA CTCACCGCGA CGTCTGTCTG GAAGTTTCTG ATCGAAAAGT  
4381 TCGACAGCGT CTCCGACCTG ATGCAGCTCT CGGAGGGCGA AGAATCTCGT GCTTTCAGCT  
4441 TCGATGTAGG AGGGCGTGGA TATGTCCTGC GGGTAAATAG CTGCGCCGAT GGTTCCTACA  
4501 AAGATCGTTA TGTTTATCGG CACTTTGCAT CGGCCGCGCT CCCGATTCCG GAAGTGCTTG  
4561 ACATTGGGGA ATTCAGCGAG AGCCTGACCT ATTGCATCTC CCGCCGTGCA CAGGGTGTCA  
4621 CGTTGCAAGA CCTGCCTGAA ACCGAAGTGC CCGCTGTTCT GCAGCCGCTC GCGGAGGCCA  
4681 TGGATGCGAT CGTGCGGCC GATCTTAGCC AGACGAGCGG GTTCGGCCCA TTCGGACCGC  
4741 AAGGAATCGG TCAATACT ACATGGCGTG ATTTTCATATG CGCGATTGCT GATCCCCATG  
4801 TGTATCACTG GCAAAGTGTG ATGGACGACA CCGTCAGTGC GTCCGTCGCG CAGGCTCTCG  
4861 ATGAGCTGAT GCTTTGGGCC GAGGACTGCC CCGAAGTCCG GCACCTCGTG CACGCGGATT



4921 TCGGCTCCAA CAATGCCTG ACGGACAATG GCCGCATAAC AGCGGTCATT GACTGGAGCG  
4981 AGGCGATGTT CGGGGATTCC CAATACGAGG TCGCCAACAT CTTCTTCTGG AGGCCGTGGT  
5041 TGGCTTGTAT GGAGCAGCAG ACGCGCTACT TCGAGCGGAG GCATCCGGAG CTTGCAGGAT  
5101 CGCCGCGGCT CCGGGCGTAT ATGCTCCGCA TTGGTCTTGA CCAACTCTAT CAGAGCTTGG  
5161 TTGACGGCAA TTTCGATGAT GCAGCTTGGG CGCAGGGTCG ATGCGACGCA ATCGTCCGAT  
5221 CCGGAGCCGG GACTGTCCGG CGTACACAAA TCGCCCGCAG AAGCGCGGCC GTCTGGACCG  
5281 ATGGCTGTGT AGAAGTACTC GCCGATAGTG GAAACCGACG CCCCAGCACT CGTCCGAGGG  
5341 CAAAGGAATA Gagtagatgc cgaccgaaca agagctgatt tcgagaacgc ctcagccagc  
5401 aactcgcgcg agcctagcaa ggcaaatgcy agagaacggc cttacgcttg gtggcacagt  
5461 tctcgtccac agttcgctaa gctcgtcgg ctgggtcgcg ggagggccgg tcgcagtgat  
5521 tcaggccctt ctggattgtg ttgggtccca gggcacgatt gtcattgcca cgcactcggg  
5581 tgatctgact gatcccgcag attggagatc gccgcccgtg cctgcccatt ggggtcagat  
5641 ctttgtgaag gaaccttact tctgtggtgt gacataattg gacaaactac ctacagagat  
5701 ttaaagctct aaggtaaata taaaattttt aagtgtataa tgtgttaaac tactgattct  
5761 aattgtttgt gtatttttaga ttccaaccta tggaactgat gaatgggagc agtggtggaa  
5821 tgcctttaat gaggaaaacc tgttttgctc agaagaaatg ccatctagtg atgatgaggc  
5881 tactgctgac tctcaacatt ctactcctcc aaaaaagaag agaaaggtag aagaccccaa  
5941 ggactttcct tcagaattgc taagtttttt gagtcatgct gtgttttagta atagaactct  
6001 tgcttgcttt gctatttaca ccacaaagga aaaagctgca ctgctataca agaaaattat  
6061 ggaaaaatat tctgtaacct ttataagtag gcataacagt tataatcata acatactgtt  
6121 ttttcttact ccacacaggc atagagtgtc tgctattaat aactatgctc aaaaattgtg  
6181 taccttttagc tttttaattt gtaaaggggt taataaggaa tatttgatgt atagtgcctt  
6241 gactagagat cataatcagc cataccacat ttgtagaggt tttacttgc ttaaaaaacc  
6301 tcccacacct cccctgaac ctgaaacata aatgaatgc aattgttgtt gttacttgt  
6361 ttattgcagc ttataatggt tacaataaa gcaatagcat cacaaattc acaataaag  
6421 catttttttc actgcattct agttgtggtt tgtccaaact catcaatgta tcttatcatg  
6481 tctggatcaa ttCGTTGCAG GACAGGATGT GGTGCCCGAT GTGACTAGCT CTTTGCTGCA  
6541 GGCCGTCCTA TCCTCTGGTT CCGATAAGAG ACCCAGAACT CCGGCCCCCC ACCGCCACC  
6601 GCCACCCCCA TACATATGTG GTACGCAAGT AAGAGTGCCT GCGCATGCCC CATGTGCCCC  
6661 ACCAAGAGCT TTGCATCCCA TACAAGTCCC CAAAGTGGAG AACCGAACCA ATTCTTCGCG  
6721 GGCAGAACAA AAGCTTCTGC ACACGTCTCC ACTCGAATTT GGAGCCGGCC GGCCTGTGCA  
6781 AAAGAGGTGA ATCGAACGAA AGACCCGTGT GTAAAGCCGC GTTTCCAAAA TGTATAAAAC  
6841 CGAGAGCATC TGGCCAATGT gcatcagttg tggtcagcag caaaatcaag tgaatCATCT  
6901 CAGTGCAACT AAAGGGGGGA TCTcgagGCC ACCATGGGTA AGCCTATCCC TAACCCTCTC  
6961 CTCGGTCTCG ATTCTACGAC TAGTGGAGGA GGAGGTTCTG GTGGTGGTGC GCTTCCAGGC  
7021 AACGGCATCT ACGTGGTGCG GGGCGAAATG GCCACCCTGA TGACGGCGAT GCGACGTGGA

7081 ACGCGTTGGA ATGCCACCGC CTACGTGGAC GACGAGAATG ACTCGCTGCT GAAGCTGTTC  
7141 ATTGACCTCA AGCATGAGCT AAATCGGATC GAGGACCTGC GTCAGATCGA GCCTCAGGTC  
7201 TTCCTGGCTC CGTTTCTTGA GGTGATTCGC ACGGCGGACG CCACGGGTCC GTTGACTIONAGT  
7261 CTAGCCTTGG CCTCGGTTAA CAAATTATTG TCTTACGGGC TAATAGATCC CACGTCTCCA  
7321 AATCTGGCTG ACATTGTGGA GCGCATTGCC GATGCTGTGA CACATGCCCG CTTTATGGGC  
7381 ACCGATCAGT CCTCGGATGG TGTCACCTTT ATGCGAGTGA TTGAGGTGCT GCACACGCTT  
7441 ATCCGCAGTC CCGAAGGAGC CGCCGTCAGC AATGTGTCCA TGTGCGAGGT GATGCTCAGC  
7501 TGCTTCAAGA TCTCCTTCGA GCCGAGGCTG AGTGAAGTGC TTCGTCGCTC GGCGGAAAAA  
7561 TCGCTCAAGG ACATGGTGCT GCTCTTCTTC ATGCGCCTTC CCCAGTTCGC CGAAGAGCGA  
7621 AGTGACACCA TGCTCCAGAA GCGATTTACT ATCGGGCGATG CTGCCAGCGG AGCTACCCAA  
7681 GAAAAACTAA AGCGTAAGAC GGTTGCCCAA GCCCAGACAG CACCCAGGAA ATCGTCAGCA  
7741 GTGGAGGAAC CGCCTCAAAC ACCGCAGTCT GCCAACCTGA CGGTGCCAGG GCACTTGAAG  
7801 GCACCCATAT TGGCCACCAC ACCCGCCAGT CCAGCGGGAA ACATATTGGA CATGCAGGGC  
7861 AAGATTACGC AGACACCAAC AACAACGGCG AGCACGGGGG AAGATGAAAC CACTGTCCCG  
7921 GAGACTCCTG TTATTCAAGT GGAGTCGACT GAATCGGAGC CCTTGCTGGA CGGCGAAACG  
7981 GGTGAAGCAA CCAGCACCTT GGCCGAGGCA AATAGTAGCG AGTACATCAA CTCGGTGGGC  
8041 GTCCGTTTTA CACAGCAGTC CACCGATCAC GATGTAACAT CACTATCCCC TTATGGCCTG  
8101 CCCTTCATCC AGGAGTTGTT CCGATTCTCT ATAATCCTCT GCAATCCTCT GGATAAGCAG  
8161 AACTCGGATA GCATGATGCA CACGGGTCTT AGTCTACTTA CTGTAGCTTT TGAAGTCGCA  
8221 GCCGATAATA TTGGAAAGTA TGAGGGCTTG CTGGAGCTGG TTAAGGACGA CTTATGCAGA  
8281 AACTTAATAT CGCTTCTCAG CTCAGAGCGG CTTAGCATCT TTGCCGCCGA TTTGCAGCTC  
8341 TGTTTTTTGC TTTTCGAGTC TCTCCGCGGA CATCTCAAGT TTCAGCTGGA AGCCTACCTT  
8401 AGAAAATTGA GCGAGATTAT TGCTAGCGAT AATCCCAAGA CGCCCTACGA AATGCGAGAA  
8461 CTCGCTCTGG ACAATCTACT GCAGTTGTGG CGCATTCCCG GCTTCGTAC GGAATTGTAT  
8521 ATCAACTACG ATTGTGACTT GTACTIONCACG GATATGTTTG AAAGTTTGAC AAACCTACTG  
8581 AGCAAGTATA CGCTGTCAGC AACGAATGCA GTTTATAGCA CCCACATTAT CTCAATGGAC  
8641 ACCCTGTAA GTGTGATAGA CAGTATCGAG CGAAATTGTG CCGCGAGCAA GAATAGCAGC  
8701 AACAACAGAG AGTCCTTGCC AGAAGCTGCC CCAGCAACAG GTGGCAGCCG CCATTCTCGC  
8761 CACAACAGCG GATTGGAGGG AATCGTAATT GATTCTGGCA ATAGTGTAGC TGCAGAAGAG  
8821 AAAGTGGAGA ACATCGCAAG CTTTATAAAT GCGAGCTCAC ATCGACTACG ACTACAATCT  
8881 GGCGGAGAGG GAGTGGGAAT AACCAGTGAA CAGCTGGCCA AGGTCAAACA GAAGAAGCGT  
8941 CTGCTATCCC AAGGCACAGA GCGATTTAAT CAGCGTCCAG AGAAAGGAAT CCAGTATCTG  
9001 CAAGAACACG GCATCCTAAA TGCCGAGCTT GATCCCATGC AGGTGGCCCT GTTCCTTCGA  
9061 GAAAATCCCG GGCTCGATAA AAAAATGATT GGCGAATATA TCTCGAAAAA GAAAACGTC  
9121 GACTCTAAGA TTCTAATTAA TTTTGTGGAC TCGTTTGATT TCACTIONGGTCT TCGAGTGGAT  
9181 CAAGCATTGC GTCTTTATCT GGAGACCTTC AGATTGCCCG GAGAGGCTCC ATTGATCTTT

9241 TTGGTGCTGG AACACTTTTC TGATCATTGG CATAAACAAA ACCAAGATCC GTTTGCCAAC  
9301 GTAGACGCTG CTTTTGCTT GGCCTATGCC ATCATCATGC TGAACATGGA TCAGCACAAAC  
9361 TCGAACGCGA AGCGTTTAAA TGTTCCAATG ACGCTCGAGG ACTTCACTAA GAATTTGCGT  
9421 GGTCTAAACG GTGGCGAAGA TTTTCGATCAA GAAATGCTGG CTCAAGTCTT TAATGCAATC  
9481 AAGAACGAAG AGATCGTTAT GCCAGCAGAG CAAACGGGTC TGGTGCCTGA AAATTATCAA  
9541 TGGAAAGTAC TGCTTCGACG AGGAGACACG CACGATGGAC ATTTTCACTA TGTGCATGAC  
9601 GCATCATACG ACGTGGAGAT CTTCAATATT GTGTGGGGTG CTTCTCTGAG CGCCCTAAGC  
9661 TTTATGTTTG ATAAAAGCAC TGAAACGGGC TACCAAAGAA CTCTAGCAGG TTTTCAGCAA  
9721 TCCGCTGCCA TATCGGCGCA CTATAATCTG CATTTCGACT TCGATGCCCT CGTTTTAACT  
9781 CTCTGCAAAT TCACAACGCT GCTGAGCAGC GTAGAACAGC ATGAGCCCCG TCCGGCGAAC  
9841 AATGAAACCC AGCAAGCTGT GAACTTTGGA TTGAACGGAA AGGCTCAGGC TGCCATGCGA  
9901 ACGGTGTTTC TATTGGTTCA CGACTACGGC GATTGCTTAA GAGAGAGCTG GAAACACATT  
9961 TTGGACCTAT ATCTGCAGCT TTTCCGTCTA AAGTTGCTGC CAAAATCATT GATCGAAGTG  
10021 GAAGACTTTT GTGAGGCGAA CGGAAAGGCC ATGTTAATCC TGGAAAAGCC CCGCGAGAAG  
10081 CAGGAATCGG GACTATTTTC CAGCCTGTAC TCATTTATCA GCTCGGAGGG TCAGCGAGAA  
10141 CCAACGTACG AGGAGCAGGA CTTTCATCAA CTGGGACGGA AGTGCATTAA GGAGTGCCAG  
10201 CTGGATCAAA TGCTGCAGGA ATCAAAGTTT GTGCAACTAG AGTCGCTGCA GGAGTTGCTT  
10261 AAATGCGTTC TAGCGCTACT GAAGGCTCCT CAGGGGCACA AATCCATTGG CCTGCCGTAC  
10321 GCCGAAGATC AAAGTGTTC CTGGATGGAA TTTTGGTCA AGATAGTTGT TCATAACCGG  
10381 GATCGCATGA TACCGCTGTG GCCAGCAGTT CGAGACCAA TGTACCTACT GCTTATGGGC  
10441 AGTGCCTCCT GTGGATACGA CTACCTACTC AACCGATGCA TTGTAGCGGT CCTAAAATA  
10501 GCTATCTATC TGATGCGAAA CGAAGAAGTGT TGTCCGATCG TATTGCAATC GCTCAAGATG  
10561 CTTTTAATGC TTAAGCCAGC CTTGTTGCTG CGCATTCTA AACAGATTC CATTGGTATC  
10621 TATGAGCTGC TCAAGACGTC GGCCCAAAT ATTCATTCCG AGCAGGACTG GCAGATTATT  
10681 TTCAATCTAC TTGAATGCGT GGGAGCCGGT GCTGTGCCG CCAATTATGA TGATGCCAG  
10741 CTGCCATTGC CGCCCAACGG AAGTGCAAAG TCTGATGGCG CTATAAGTGG CGAAGAGGAC  
10801 GCAACTGCCG TGCCAGAGCG TGGTTACTC TCGGATTCGG AGATCACGAA AGCATCTGCA  
10861 GCACCTGCAG TCTCCAGTCC AAGTGCTGAG AACTGGATTC TGGTCAATAA CAAGGACAGT  
10921 GAATTGACTA CGGCCTCTAG ACCACAATCT CCGCCTAGCC TGAGTGCTCC TCCAGTAAAT  
10981 ACGCTTGTGT ACAATTGCCA GCTACTAGAC CACGCTCCGT TTGCTCTTT CAAGTGCTGG  
11041 GATTGCTGG CGTTTATCGT GCGCAGTGTG GCACACATCA CGCCTTACAA TTTTGAAGCC  
11101 TCGTTCGCT GCATCCGCAT CTTTGTGGAG GCTTGTGGG ATGGAGGTAT ACGCCAGCGG  
11161 CGAAAGCTGG AATCGGCGG TAAGCAGAAA AGTTCCAAGA AGCGCAGCGA ACGCAAACCG  
11221 GGCATGGCTT CCTCCGCCTC GAGTAGTAAT CTTACTCTTC TGACGGGCGA CCCGTCCGAC  
11281 AACAGATAA ACGGAAATGC GGCAGAGCAG GAGGACCTGG CCCAGCGCTA CGAACAGTTG  
11341 TCCATTCAAC TGCTGGACCT GATGTATACG TTGTACACGC GAACTGCCA AATCTTCCGA

11401 TGGTGGGCGG AAGAAGGATG CACAGTGCCG CAGTCGGCAG CTTTGTGGTC ACCGGGCTGG  
11461 TGTCATTGC TTCAGGAAT CGCCAGGCTG GCAATGGATC GACGGCGAGA GGTGCGCACC  
11521 CATGCCATAT CGTGCCTGCA GCAGCGGGCA TTGCTAGTCC ATGACCTGCA AACGTTGTCTG  
11581 GGAACGGAGT GGTGCTCTTG CTTCCACCAG GTGCTGTTC CCCTCCTAAA CGAACTGCTG  
11641 CCCGAGAGTA ATGCAGCCGG CCAACTGGAT GCCGCTCTCC TCGAAGAGTC GCGTATACGA  
11701 ACGGCCACCA TTATGTCTAA GGTGTTCTCG CAACACCTGA CCACGCTCAT CGAGCTGGGA  
11761 AATGCTTTTA ACGAGCTGTG GCTGGATATA TTGGACTACA TTGAGCGCTT TATGAAGGTG  
11821 GGATCGGACA CATTGTCCGA GCAGATGCAG GAGATACTGA AGAACATGCT GCTGGTATG  
11881 CATTAGTGC GAGTGTTC CAATCAGGAT GGTAGTTTAC AGCAGGCTCT TTGGGAGCTA  
11941 ACCTGGCGAC GCATCGGCGA ATTTTTGCC AACCTGAAGG AGGAGCTTTT CCACGACGAA  
12001 GGCAAGCGAG CTCAGACCTT AACGAACCCA GCTCCACAGG CAGCTGTGGC TGCCGCTCCA  
12061 CAGCAACAGT TACCAGCGGT GACCATTTTG CCCAGGCAAA CCCAGGTTTC CAACGAGTTA  
12121 GTGGTGAGCG CGCCTACTCC GCCGGCAGCC ACACCTTTC TGGGCTCTCC CGTCGAGTCG  
12181 CCGAGGCGGA GCATAATACT GCAGCCACCC ATGGCCGATG TACTGCAACA GCCGCCAGC  
12241 TTTGTATTTG CTCAGCCCAT TATTGTGCCA CCCCAGCCGC CTGCAGTTAC GGACCCAATA  
12301 CCACCAAGTA CATTATTGCC GGATTTGGTG AATGAGGCAA CTGCTGCTGC CGTGCAAGCC  
12361 ACGACCACGT CCCCAGCGCA CAGCCCGCAG GAGGCGGAGC AGCCGGCTTC AATAGTGAG  
12421 CAGACCAACA TCGTAACCAC CAACAATACG TACAATAGCT ACGCCATTGA GGTGCCCATG  
12481 GCGCCGAGGA CAACTGCGGA ACAGTTTGGG CAGCAGCAGC AGCAACTGCT TTACCAACAG  
12541 TACTATCAAC AGTATCAGGC CCAGCAGCAA CAGTTGCCGG CTCCAGCCAG CGACCCTGCC  
12601 ATCAATGTGC CAATTAGTCA TCTGCTGGCC GGAAATGCGT ACCCCTCGCT CCCCAAAATG  
12661 CCGCAGGCAT CCATTGTGCA CAGCTTTGCG CCCGTTTACG AAAGCCAGGC GGCGACGAGT  
12721 GGAGCTGGGA CAGCAGCAGC GGACATCTAT CAGGAGTATG TGCAAAATCC GTACAACCTT  
12781 ACGTTGCAAC AGCATCCCCA ACAGCAGCTC CATCAGCAGC AGCAGCAGCA ACAACAGCAG  
12841 GCTACAGGAA TGGCCAACGC ATTTCCCGCC GTTGCCACGC CAGCCAATA CTTAATGTG  
12901 AATGTGGACC CCAGTAGCAT ACCGCCCGGA TCGGAACTGC TCTACGGCCA GCAGTAATtc  
12961 tagtcgacca tgaagatcaa gatcattgcc ccgccagagc gcaagtactc tgtctggatc  
13021 ggtggctcca tcctggCTTC GCTGTCCACC TTCCAGcaga tgtggatctc caagcaggag  
13081 tacgacgagt ccggcccctc cattgtgcac cgcaagtgct tctaagaagg atcgcttgtc  
13141 tgggcaagag gatcaggatc gggatggtct tgattctgct ggaggaggag gaggagaagt  
13201 cgaggaagca gcagcgaag tgcaagtgcg agtggtgga gtttgagtg cagcacaaca  
13261 aaatcaaca caacaccaac tacaagatga aaagagcgga accacctgca caccatcatc  
13321 actatcatca tcgttttggg cgcattgtgt gtggttcag cgtattaata taattaattt  
13381 attccacatg agatatgata tgatatacta tgtatTTTTT gTTTTTTTT tatttgtaaa  
13441 ctttaatat aacaagaact acaaaaaatg aaaatgagcg aaaatgcata ttctgccatt  
13501 ccacacacac accaacaaca ccaacacac gcacacccac aagcttacac acacacattc

```

13561 gcggcatgac aaggacatca agataaagaa gaacttaaag aagatatttc ccaaagcgca
13621 aaaagaacac acacacattg caaacacaaa acaacacact agcgttttgt acaattcgtc
13681 agcaacctta tgtattatth tttattatg atgtaattat aaacaaagtg aaacaaaaat
13741 atgaaaacaa aaaggaaaat caaatctgtc ttctctttct cccgctctcc tcgctctctg
13801 ctgctaacct cgccctctcc tctctcatct ttttgtctgt ctctcttcca cttttttgcc
13861 ggccggcaaa ataataaccc acacacactc acacttggct gcagtttcgc gtgcatatt
13921 cacacacatt caagcataca agcatacata catatgtatt tttttttat ttgtacactt
13981 ttctaattgc atgcgtatcg attgataagt ttacgcctga aatgttaat taaaatgtga
14041 aatgcaact gaaaaactga tgaatgaaa caacaacaag cgaacaattt gctacatgtg
14101 tattgtctaa caaccgttac tgcaacggtt gcttcggaaa aggggtgaaga ggaagagggc
14161 acgaggtcga ctagagcggc cgccgacgcg aggctggatg gccttcccca ttatgattct
14221 tctcgcttcc ggcggcatcg ggatg

```

//

**pMK-V5::Sec71<sup>E677K</sup>**

```

LOCUS      pMK_V5_Sec71_E677K      13255 bp ds-DNA      circular      27-FEB-
2020
COMMENT    pMK-V5-garz from 1 to 14245
COMMENT    pMK33-CFH-BD from 1 to 8550
COMMENT    >pMK33-CFH-BD, 8550 bases.
COMMENT    ApEinfo:methylated:1
FEATURES   Location/Qualifiers
    misc_feature   6493..6860
                /label=MT-promoter
                /ApEinfo_fwdcolor=cyan
                /ApEinfo_revcolor=#00ff00
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
                width 5 offset 0
    misc_feature   4326..5351
                /label=Hyg
                /ApEinfo_fwdcolor=#ffffcc
                /ApEinfo_revcolor=#00ff00
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
                width 5 offset 0
    misc_feature   6979..6984
                /label=SpeI
                /ApEinfo_fwdcolor=#ccff66

```



```

    /ApEinfo_revcolor=#ffffcc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
rep_origin    complement(777..1459)
    /label=ColE1 origin
    /ApEinfo_fwdcolor=gray50
    /ApEinfo_revcolor=gray50
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind   5645..5664
    /label=pUAST-R
    /ApEinfo_fwdcolor=#0a00ff
    /ApEinfo_revcolor=#0a00ff
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
CDS           complement(1557..2216)
    /label=AmpR
    /ApEinfo_fwdcolor=yellow
    /ApEinfo_revcolor=yellow
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind   complement(2135..2159)
    /label=Amp-GF
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
polyA_signal  complement(6293..6484)
    /label=SV40 late polyA
    /ApEinfo_fwdcolor=pink
    /ApEinfo_revcolor=pink
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind   2528..2545
    /label=pQE60-F
    /ApEinfo_fwdcolor=#ff00bd
```

```
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    1677..1700
    /label=Amp-GR1
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    6937..6978
    /label=V5
    /ApEinfo_fwdcolor=#ffffcc
    /ApEinfo_revcolor=#ffffcc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    6985..7008
    /label=Linker GL3
    /ApEinfo_fwdcolor=#ffcc66
    /ApEinfo_revcolor=#ffcc66
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    complement(12340..12359)
    /label=Act5C-qR2
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    12144..12163
    /label=Act5C-qF1
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    complement(12351..12370)
    /label=Act5C-qR1
    /ApEinfo_fwdcolor=#ff00bd
```

```

    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind    12078..12097
    /label=Act5C-qF2
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind    complement(12047..12065)
    /label=Seq-MK-R
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature   12006..12027
    /label=New Feature
    /ApEinfo_fwdcolor=#ffffcc
    /ApEinfo_revcolor=#ffffcc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature   6928..6928
    /label=BamHI
    /ApEinfo_fwdcolor=#ffcc66
    /ApEinfo_revcolor=#ffcc66
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature   6896..6913
    /label=Metallothionein_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind    6836..6853
    /label=SEQ-MT-F2
    /ApEinfo_fwdcolor=#ff00bd
```

	<pre>/ApEinfo_revcolor=#ff0003 /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0} width 5 offset 0</pre>
CDS	<pre>7009..11964 /label=Sec71 /ApEinfo_fwdcolor=#99ccff /ApEinfo_revcolor=#cde7f7 /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0} width 5 offset 0</pre>
CDS	<pre>9885..9885 /label=Sec71-PA /ApEinfo_fwdcolor=#99ccff /ApEinfo_revcolor=#cde7f7 /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0} width 5 offset 0</pre>
CDS	<pre>7972..7974 /label=Sec71-PA(1) /ApEinfo_label=Sec71-PA /ApEinfo_fwdcolor=#99ccff /ApEinfo_revcolor=#cde7f7 /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0} width 5 offset 0</pre>
misc_feature	<pre>11562..11562 /label=T&gt;G silent /ApEinfo_fwdcolor=cyan /ApEinfo_revcolor=#00ff00 /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0} width 5 offset 0</pre>
misc_feature	<pre>11358..11358 /label=T&gt;C silent /ApEinfo_fwdcolor=cyan /ApEinfo_revcolor=#00ff00 /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0} width 5 offset 0</pre>
misc_feature	<pre>10504..10525 /label=Chang-F1</pre>

```

    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    9765..9784
    /label=sec71-GF6
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    7563..7582
    /label=sec71-GF3
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature   9885..9885
    /label=A>C silent
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature   9624..9624
    /label=A>G in 40A
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
CDS            8064..8064
    /label=Sec71-PA(2)
    /ApEinfo_label=Sec71-PA
    /ApEinfo_fwdcolor=#99ccff
    /ApEinfo_revcolor=#cde7f7
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
CDS            8595..8595
```



```
/label=Sec71-PA(3)
/ApEinfo_label=Sec71-PA
/ApEinfo_fwdcolor=#99ccff
/ApEinfo_revcolor=#cde7f7
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
protein_bind 8728..9300
/label=Sec7 domain
/ApEinfo_fwdcolor=#0080ff
/ApEinfo_revcolor=#0080ff
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature 11442..11442
/label=G>A silent
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature 9145..9147
/label=S191
/ApEinfo_fwdcolor=#66ff66
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature 11547..11547
/label=C>T silent
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature 11746..11746
/label=T>C silent(1)
/ApEinfo_label=T>C silent
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
```

```
width 5 offset 0
primer_bind    8545..8564
               /label=Sec71-GF5
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind    9245..9268
               /label=Sec71-DRSC01893-F (8A10)
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind    complement(9721..9756)
               /label=Sec71-DRSC01893-R
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature   11319..11319
               /label=T>C silent(2)
               /ApEinfo_label=T>C silent
               /ApEinfo_fwdcolor=cyan
               /ApEinfo_revcolor=#00ff00
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind    11049..11068
               /label=sec71-GF8
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind    10504..10525
               /label=sec71-GF7
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
```

```
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    complement(11219..11240)
    /label=Chang-R1
    /ApEinfo_fwdcolor=#fb53d0
    /ApEinfo_revcolor=#fc5a5d
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    7919..7938
    /label=sec71-GF4
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    complement(10557..10578)
    /label=Sec71-GR3
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    9034..9036
    /label=E677 (E740 in garz)
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    8595..8595
    /label=G>A silent(1)
    /ApEinfo_label=G>A silent
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    8064..8064
    /label=C>T confirmed in 40A 2013
    /ApEinfo_fwdcolor=cyan
```

```

/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 9142..9144
/ApEinfo_label=F190 F190Y=BFA-hypersensitive
/ApEinfo_label=F713Y(BFA-sensitive)
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 9552..9552
/ApEinfo_label=T>C in 40A
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 9663..9663
/ApEinfo_label=A>C in 40A
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 9714..9714
/ApEinfo_label=A>G 40A
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 11331..11331
/ApEinfo_label=T>C in 40A(1)
/ApEinfo_label=T>C in 40A
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind 11657..11674
```

```
    /label=Sec71-GF10
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    7035..7052
    /label=GF9
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    9192..9210
    /label=Sec71-GF11
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    complement(9370..9393)
    /label=Sec71-GR4
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    8792..8815
    /label=Sec71-GF12
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    10132..10150
    /label=Sec71-GF13
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    9154..9156
```



```
    /label=M717(M194 in sec7 domain M>L = BFA-resistant)
    /label=M717L(BFA-resistant)
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    11965..11967
    /label=STOP
    /ApEinfo_fwdcolor=#66ccff
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    8898..8920
    /label=Sec71-GF14
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    9068..9089
    /label=Sec71-GF15
    /ApEinfo_fwdcolor=#fb53d0
    /ApEinfo_revcolor=#fc5a5d
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    complement(9094..9116)
    /label=Sec71-crRNA7(Protospacer)
    /ApEinfo_fwdcolor=#ccff66
    /ApEinfo_revcolor=#ffff9f
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    9094..9096
    /label=PAM
    /ApEinfo_fwdcolor=#fc81f0
    /ApEinfo_revcolor=#fc81f0
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
```

```
misc_feature 9183..9202
    /label=Sec71-crRNA8 Protospacer
    /ApEinfo_fwdcolor=#ccff66
    /ApEinfo_revcolor=#ffffcc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature 9203..9205
    /label=PAM(1)
    /ApEinfo_label=PAM
    /ApEinfo_fwdcolor=#fc81f0
    /ApEinfo_revcolor=#fc81f0
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature 9823..9823
    /label=T>C Val>Ala in FRT40A
    /ApEinfo_fwdcolor=#ccff66
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
```

ORIGIN

```
1 cccgcgttgc aggccatgct gtccaggcag gtagatgacg accatcaggg acagcttcaa
61 ggatcgctcg cggctcttac cagcctaact tcgatcattg gaccgctgat cgtcacggcg
121 atttatgccg cctcggcgag cacatggaac gggttggcat ggattgtagg cgccgcccta
181 taccttgtct gcctccccgc gttgcgtcgc ggtgcatgga gccgggccac ctcgacctga
241 atggaagccg gcggcacctc gctaacggat tcaccactcc aagaattgga gccaatcaat
301 tcttgcgag aactgtgaat gcgcaaacca acccttgca gaacatatcc atcgcgtccg
361 ccatctccag cagccgcacg cggcgcattc cgggcagcgt tgggtcctgg ccacgggtgc
421 gcatgatcgt gtcctgtcgc ttgaggacc cggctaggctg gcgggggttc cttactggtt
481 agcagaatga atcaccgata cgcgagcga cgtgaagcga ctgctgctgc aaaacgtctg
541 cgacctgagc aacaacatga atggctcttc gtttccgtgt ttcgtaaagt ctggaaacgc
601 ggaagtcagc gctcttccgc tcctcgtc actgactcgc tgcgctcggc cgttcggctg
661 cggcgagcgg taccagctca ctcaaaggcg gtaatacggc tatccacaga atcaggggat
721 aacgcaggaa agaacatgtg agcaaaaggc cagcaaaagg ccaggaaccg taaaaggcc
781 gcggttctgg cgtttttcca taggctccgc cccctgacg agcatcaca aaatcgacgc
841 tcaagtcaga ggtggcgaaa cccgacagga ctataaagat accaggcgtt tcccctgga
901 agctccctcg tgcgctctcc tgttccgacc ctgccgctta ccggatacct gtccgccttt
```

961 ctcccttcgg gaagcgtggc gctttctcat agctcacgct gtaggtatct cagttcggtg  
1021 taggtcgttc gctccaagct gggctgtgtg cacgaacccc ccgttcagcc cgaccgctgc  
1081 gccttatccg gtaactatcg tcttgagtcc aaccggtaa gacacgactt atcgccactg  
1141 gcagcagcca ctggtaacag gattagcaga gcgaggatg taggcgggtg tacagagttc  
1201 ttgaagtggg ggcctaacta cggctacact agaaggacag tatttgggat ctgcgctctg  
1261 ctgaagccag ttaccttcgg aaaaagagtt ggtagctctt gatccggcaa acaaaccacc  
1321 gctggtagcg gtggtttttt tgtttgcaag cagcagatta cgcgcagaaa aaaaggatct  
1381 caagaagatc ctttgatctt ttctacgggg tctgacgctc agtggaacga aaactcacgt  
1441 taagggattt tggatcatgag attatcaaaa aggatcttca cctagatcct tttaaattaa  
1501 aatgaagtt ttaaatcaat ctaaagtata tatgagtaaa cttggctgca cagttaccaa  
1561 tgcttaatca gtgaggcacc tatctcagcg atctgtctat ttcgttcac catagttgcc  
1621 tgactccccg tcgtgtagat aactacgata cgggagggtt taccatctgg cccagtgct  
1681 gcaatgatac cgcgagacc acgctcaccg gctccagatt tatcagcaat aaaccagcca  
1741 gccggaaggg ccgagcgcag aagtggctct gcaactttat ccgcctccat ccagtctatt  
1801 aattgttgcc gggaaactag agtaagtagt tcgccagtta atagtttgcg caacgttgtt  
1861 gccattgctg caggcatcgt ggtgtcacgc tcgtcgtttg gtatggcttc attcagctcc  
1921 ggttcccaac gatcaaggcg agttacatga tccccatgt tgtgcaaaaa agcggttagc  
1981 tccttcggtc tccgatcgt tgtcagaagt aagttggccg cagtgttacc actcatggtt  
2041 atggcagcac tgcataattc tcttactgtc atgccatccg taagatgctt ttctgtgact  
2101 ggtgagtact caaccaagtc attctgagaa tagtgtatgc ggcgaccgag ttgctcttg  
2161 ccggcgtcaa cacgggataa taccgcgcca catagcagaa ctttaaaagt gctcatcatt  
2221 ggaaaacggt cttcggggcg aaaactctca aggatcttac cgctgttgag atccagttcg  
2281 atgtaacca ctcgtgcacc caactgatct tcagcatctt ttactttcac cagcgtttct  
2341 gggtgagcaa aaacaggaag gcaaaatgcc gcaaaaaagg gaataagggc gacacggaaa  
2401 tgttgaatac tcatactctt ctttttcaa tattattgaa gcatttatca gggttattgt  
2461 ctcatgagcg gatacatatt tgaatgtatt tagaaaaata acaaaatagg ggttccgcgc  
2521 acatttcccc gaaaagtgcc acctgacgtc taagaaacca ttattatcat gacattaacc  
2581 tataaaaaata ggcgtatcac gaggcccttt cgtcttcaag aattcacatt tgtacgaatt  
2641 ttttttttat caaaagttcg agtttttcac caatttcctc atcaaccgag caaggcaaac  
2701 ggctttgaat aatattggtg tatatataca tatatcaaat cgctgctgac tgcgtgattg  
2761 atggcccaa gattacatat tatcgaatca ggattcagaa ggagatcaat gtcaaatgcg  
2821 gacaggaaca tgaagacgc ctgttatgcy caattaaaaa tttgggttta attgctgtgg  
2881 aaactgttgt tggcggcatc ttaagttcct gtttaacaac atcaactact tatgtacgta  
2941 gaagcgttta agccatttgc atacagatga gaactggctt ttgtgctaata cagtcaagat  
3001 gactccgatg atgatgactc attacctgac cagttttcgc tgctttcttt tcaacaacta  
3061 cttgtatatg tattgtatcc aatagcaata cattgaattt ccatggctca gtcacgtatt

3121 atcatttaat tgacaccaag tcgtgttatt gttgagctat cgagttcagc tcaaacattt  
3181 cttattccca tgaataagcc ggcaaaaata tgcaatctat gaaagttaat ataagcaaac  
3241 cttactttga ctcaatacca atgcactttg tgtc gatagg ttcacgcaat tgaggcgatt  
3301 attccgataa cccaagcgat tgactgttcc cgtttcgatt ccaattgaaa tttggaaatg  
3361 tacaatagtt ttgctatatg ctgtcaagta cgctcttata ttctctgggt tttcttcaga  
3421 gtttcgaaac gcttcttctt ttttttgttt tttttttttt ggaatctcgt attttggaag  
3481 gggctcccct ctggaatttg ttacactgtc gttatcattg cgaacaagcg gcccgaaagct  
3541 atcagcgact ttaacattta caatgcactt ttttacgacc aattaaatgt acattttcct  
3601 ttcttcgccc gttgataagc gaacgcgatg tggcgcaggc aatgtgttg ccttgcgaca  
3661 caaacgcaat caaaatggat tcaatttcgc tttttccag tgaaacgaag aacgaaccga  
3721 ccatcatgat atgctcctct gcatgttgcg tattgaatca atgacaattt caattaagcc  
3781 gcccgttcgt catgctgttt cggtgccttc gaaatgctga taacgctgct gtcctcaac  
3841 tgctttgcat gtggacacaa ttccatttat ttaattcttt tatttggatc ggttaaatta  
3901 aaaagcgcct tgttacgcat ttaacgttgt ttccggtgcg tgggtggttc atgcttctgg  
3961 gaacggcaaa tgggtttagg attgggaacc cctcatcatt tggttggaata tactattcaa  
4021 cctacaaaag taacgttaaa caacactact ttatatttga tatgaatggc cacacctttt  
4081 atgccataaa acatattgta agagaatacc actcttttta ttccttcttt ccttcttgta  
4141 cgttttttgc tgtaagtagg tcgtggtgct ggtgttgtag ttgaaataac ttaaaatata  
4201 aatcataaaa ctcaaacata aacttgacta tttatttatt tattaagaaa ggaaatataa  
4261 attataaatt acaacagggt atggacctgc agccaagctt ggcgctcgtc cgggggcaat  
4321 gagatatgaa aaagcctgaa ctacccgca cgctctgca gaagtttctg atcgaagagt  
4381 tcgacagcgt ctccgacctg atgcagctct cggagggcga agaattcctg gctttcagct  
4441 tcgatgtagg agggcgtgga tatgtcctgc gggtaaatag ctgcgccgat ggtttctaca  
4501 aagatcgtaa tgtttatcgg cactttgcat cggccgcgct cccgattccg gaagtgttg  
4561 acattgggga attcagcgag agcctgacct attgcatctc ccgccgtgca cagggtgtca  
4621 cgttgcaaga cctgcctgaa accgaactgc ccgctgttct gcagccggtc gcggaggcca  
4681 tggatgcat cgctgcggcc gatcttagcc agacgagcgg gttcggcca ttcggaccgc  
4741 aaggaatcgg tcaatacact acatggcgtg atttcatatg cgcgattgct gatccccatg  
4801 tgtatcactg gcaaaactgt atggacgaca ccgtcagtgc gtccgtcgcg caggctctcg  
4861 atgagctgat gctttgggcc gaggactgcc ccgaagtccg gcacctcgtg cacgcggatt  
4921 tcggctcaa caatgtcctg acggacaatg gccgcataac agcggtcatt gactggagcg  
4981 aggcgatgtt cggggattcc caatacagag tcgccaacat cttcttctgg aggccgtggt  
5041 tggcttgat ggagcagcag acgcgctact tcgagcggag gcatccggag cttgcaggat  
5101 cgccgaggct ccgggcgtat atgctccgca ttggtcttga ccaactctat cagagcttgg  
5161 ttgacggcaa tttcgatgat gcagcttggg cgcagggctg atgcgacgca atcgtccgat  
5221 ccggagccgg gactgtcggg cgtacacaaa tcgcccgcag aagcgcggcc gtctggaccg

```
5281 atggctgtgt agaagtactc gccgatagtg gaaaccgacg ccccgact cgtccgaggg
5341 caaaggaata gagtagatgc cgaccgaaca agagctgatt tcgagaacgc ctacagccagc
5401 aactcgcgcg agcctagcaa ggcaaatgcg agagaacggc cttacgcttg gtggcacagt
5461 tctcgtccac agttcgctaa gctcgtcgg ctgggtcgcg ggagggccgg tcgcagtgat
5521 tcaggccctt ctggattgtg ttggtcccca gggcacgatt gtcatgcca cgcactcggg
5581 tgatctgact gatcccgcag attggagatc gccgcccgtg cctgcccatt ggggtcagat
5641 ctttgtgaag gaaccttact tctgtggtgt gacataattg gacaaactac ctacagagat
5701 ttaaagctct aaggtaaata taaaattttt aagtgtataa tgtgttaaac tactgattct
5761 aattgtttgt gtatttttaga ttccaaccta tggaactgat gaatgggagc agtggtgga
5821 tgcctttaat gaggaaaacc tgttttgctc agaagaaatg ccatctagtg atgatgaggc
5881 tactgctgac tctcaacatt ctactcctcc aaaaaagaag agaaaggtag aagaccccaa
5941 ggactttcct tcagaattgc taagtttttt gagtcatgct gtgttttagta atagaactct
6001 tgcttgcttt gctatttaca ccacaaagga aaaagctgca ctgctataca agaaaattat
6061 ggaaaaatat tctgtaacct ttataagtag gcataacagt tataatcata acatactgtt
6121 ttttcttact ccacacaggc atagagtgtc tgctattaat aactatgctc aaaaattgtg
6181 taccttttagc tttttaattt gtaaaggggt taataaggaa tatttgatgt atagtgcctt
6241 gactagagat cataatcagc cataccacat ttgtagaggt tttacttgc ttaaaaaacc
6301 tcccacacct ccccctgaac ctgaaacata aatgaatgc aattgttgtt gttacttgt
6361 ttattgcagc ttataatggt tacaataaa gcaatagcat cacaaattc acaataaag
6421 catttttttc actgcattct agttgtggtt tgtccaaact catcaatgta tcttatcatg
6481 tctggatcaa ttcgttgcag gacaggatgt ggtgcccgat gtgactagct ctttgctgca
6541 ggccgtccta tcctctggtt ccgataagag acccagaact ccggccccc accgccacc
6601 gccacccca tacatatgtg gtacgcaagt aagagtgcct gcgcatgcc catgtgccc
6661 accaagagct ttgcatccca tacaagtccc caaagtggag aaccgaacca attcttcgcg
6721 ggcagaaca aagcttctgc acacgtctc actcgaattt ggagccggc ggcgtgtgca
6781 aaagaggtga atcgaacga agaccctgt gtaaagccgc gtttccaaa tgtataaac
6841 cgagagcatc tggccaatgt gcatcagttg tggtcagcag caaatcaag tgaatcatct
6901 cagtgcaact aaagggggga tctcgaggcc accATGGGTA AGCCTATCCC TAACCCTCTC
6961 CTCGGTCTCG ATTCTACGAC TAGTGGAGGA GGAGGTTCTG GTGGTGGTCA CAACAACTCC
7021 AAAAAACCA AGGAAATGTT CATCGTGCCT GCTCTAGAAA AGATCCTTGC CGATAAGGAC
7081 ATACGGCGCT CCCATCACTC GCAGCTGAAG AAGTCCTGCG ATTCGGCGCT GGAGCAGATT
7141 AAGGCGGAGC TAATCAGTGC CGGCCAGATC GCAGAGGGCA ATGAGCTGCC CTGTGCCGCA
7201 CTCCCCTGTC CCAAGAATGA TGCAGCGAGC ATCATAAATG CGGAGACCTA CTTTCTCCCC
7261 TTCGAGCTTG CCTGCAAGAG CCGCTCGCCC AGGATCGTGG TCACCGCACT GACTGCCTG
7321 CAGAACTCA TTGCTATGG CCATTTGACA GGATCCATTC AGGACTCGGC CAATCCGGGT
7381 CACCTGCTCA TCGACCGTAT CGTTGTGACC ATATATGGCT GCTTCAGTGG TCCCCAGACG
```



7441 GACGAGGCCG TCCAAGTCA GATAATAAAG GCTCTGCTCA CGGTGGTCAC CTCGCAGCAT  
7501 GTGGAAATCC ATGAATTCAC ACTGCTGCAA GCTGTGCGCA CCTGCTACGA CATCTATTTG  
7561 TCCAGCAAGA ACCTGGTCAA TCAGACCACA GCACGCGCTA CGCTCACCCA AATGTTGAAC  
7621 GTGATATTTG CCCGCATGGA GAATCAAGTG TACGAGCTAC CACCTCCCAA TTCCAATCCC  
7681 ACCAACGGCA GCATCCACTC GGAGGATTGC AATGGCTCGG GAGAGGAGTC GCTGCGGGAT  
7741 TCCGACGAAG TAATTGCCTC GGAAGTCTG GCGGAGATCA TATCAGCTGC CTACAATGAG  
7801 GCGATGAAGG ATCAGGAATC GGTCGGTGAG CCAGAGCCAA CACTTAATGG AAACGACTAC  
7861 TCCTCGCACT CGGATCACGA CAGTGTGGAG CTGCACAGCG AAAACGATGC GGTTGTAACG  
7921 GCTAAGTTTA CGCACATCCT GCAGAAAGAT GCTTTTCTCG TGTTCCGGGC ACTGTGCAAG  
7981 CTATCGATGA AGCCTTTGCC GGATGGACAT CCAGATCCGA AATCGCACGA GCTGCGTTCC  
8041 AAGGTGCTGT CATTGCATCT GCTGCTGCTC ATCCTCCAGA ATGCCGGGCC CGTCTTCCGC  
8101 TCCAACGAGA TGTTTCATCAT GGCCATTAAG CAGTACCTGT GCGTGGCCTT GTCAAACAAC  
8161 GGAGTCAGTC TGGTGCCGGA GGTCTTCGAG CTGTGCTTT CAATCTTCGT TGCCCTACTC  
8221 TCGAACTTCA AGGTGCATCT TAAGCGGCAG ATAGAGGTGT TCTTCAAGGA AATCTTCTTA  
8281 AACATTCTTG AGGCGAACTC AAGCAGCTTC GAGCACAAT GGATGGTAAT CCAAGCGCTG  
8341 ACACGTATTT GTGCTGACGC CCAGTCCGTG GTGGATATCT ATGTTAATTA CGATTGCGAC  
8401 TTTTCGGCTG CAAACCTTTT TGAGAGACTG GTCAACGATC TTTGAAAAT TGCCCAGGGT  
8461 CGTCAGGCTC TCGAACTGGG CGCCAATCCG ATGCAAGAGA AATCGATGCG CATTGCGGGC  
8521 CTGGAGTGTC TTGTCTCCAT TCTTAAGTGC ATGGTAGAGT GGAGTAAGGA CTTGTATGTT  
8581 AATCCAAACA TGCCGGTTCC ACCTATGCAA GTCCAATCGC CGACAAGCAC TGAGCAGGAT  
8641 CAGGCGGACA CAACTATCCA AACGATGCAC AGTGGTTCCA GTCATAGTTT GAACTCCAAT  
8701 CAGGAGCAAC TACAGGATCT TCCCGAGGCA TTGGAGGAGC GCAAGATGCG CAAGGAAGTG  
8761 ATGGAACAG GCATTGAGTT ATTCAATCGT AAGCCTCAGA AAGGAGTGCA ATTCCTGCAG  
8821 GAGAAGCAGT TGCTGGGTGC CACATGCGGG GACATTGCGC GCTGGCTGCA CGAGGACGAA  
8881 CGACTGGACA AGACAGTGAT CGGAAACTAC ATTGGCGAGA ATGACGACCA CTCCAAGGAA  
8941 GTGATGTGCG CTTACATCGA TGCTTTGAC TTTGCCCCAA TGGAGGTGGT GGCCGCTTG  
9001 AGATTTCTTC TCGAGGGGTT CCGCCTGCCA GGAaAAGCAC AAAAAATCGA TCGGCTGATG  
9061 GAGAAGTTCC CCAGTAGATA CTGCGAATGC AATCCGAAGA ACCAGCTATT CCAAAGCGCA  
9121 GACACCGTCT ACGTGCTGGC ATTCAGCATC ATTATGCTGA CCACGGATCT TCATTGCGCCG  
9181 CAGGTCAAGC ACAAGATGAC CAAGGAGCAG TACATTAATA TGAACCGCGG CATCAGCGAC  
9241 AGCAAGTCCG ATTTGCCCGA GGAGTACTTG TCGTCCATCT ACGACGAGAT TTCTGAACAC  
9301 GAAATTAAGA TGAAGAACAA CTCCGGTATG CTTCAACAGG CGAAACCCAC TGGAAGCAG  
9361 GCCTTCATAA CGGAGAAACG CAGAAAGCTG TTGTGGAACA TGGAGATGGA GGTCATCTCG  
9421 CTGACGGCCA CCAATCTAAT GCAGTCAGTT TCGCACGTCA AGTCACCCTT CACCTCAGCG  
9481 AAACACTTGG AGCATGTCCG GCCCATGTTC AAAATGGCTT GGACACCATT TCTGGCCGCT  
9541 TTCTCTGTGG GTCTCCAGGA CTGCGACGAT CCTGAGATTG CTACACTCTG CTTGGATGGT

9601 ATACGTTGTG CTATTCGAAT CGCATGCATC TTCCACATGT CCCTGGAGCG AGATGCCTAT  
9661 GTACAAGCCC TGGCCAGGTT TACTCTCCTG AATGCTAACT CGCCCATCAA CGAAATGAAG  
9721 GCCAAGAATA TCGATACCAT CAAGACGCTT ATAATGGTAG CCCACACGGA TGGCAATTAT  
9781 CTGGGCAGCA GCTGGCTGGA TATAGTGAAG TGCATTAGCC AGTTGGAGCT GGCCCAACTG  
9841 ATCGGCACTG GGGTGC GGCC CCAGTTTCTT TCTGGAGCGC AGACAACGCT CAAGGACTCG  
9901 CTTAATCCCA GCGTGAAAGA ACACATCGGC GAGACGAGCA GCCAGAGCGT GGTGGTTCGCA  
9961 GTCGATCGTA TTTTCACCGG CTCAATGCGA CTGGATGGCG ATGCTATCGT GGACTTCGTG  
10021 AAGGCCCTGT GCCAGGTGTC TGTGGATGAG CTTTCAGCAGC AGCAACCGAG GATGTTCTCC  
10081 TTGCAAAAGA TAGTGAAAT TAGTTACTAC AACATGGAGC GTATTCTGTCT GCAGTGGTCA  
10141 CGCATTTGGC AAGTTTTGGG TGAGCACTTT AACGCGGTGC GATGCAATAG CAACGAGGAG  
10201 ATCTCATTTT TCGCCCTGGA CTCACTGCGT CAGTTGTCGA TGAAGTTCAT GGAGAAGGGC  
10261 GAGTTCAGTA ATTTCCGCTT CCAGAAGGAT TTCCTGCGTC CCTTTGAGCA TATCATGAAG  
10321 AAAAACGCAT CGCCGGCAAT ACGAGATATG GTGGTGCCTG GCATTGCCCA GATGGTAAAC  
10381 TCACAGGCGC ATAACATCCG TTCCGGCTGG AAGAATATCT TTAGCATTTT CCACCTGGCA  
10441 GCGGGAGACA ACGAAGAGCC AATTGTGGAG CTGGCCTTCC AAACCACGGG CAAAATCATC  
10501 GGTGATCTGT ACAAGCGTCA GTTCGCCATT ATGGTGGACT CGTTCCAGGA TGCGGTCAAG  
10561 TGCCTGTCAG AGTTCGCCAC CGCCAGATTC CCCGATACCA GCATGGAAGC CATACTGTG  
10621 GTCGTACCT GCGCGCAGTG CGTCCACGAG GCACCACAAC TGTTTGCGGA GCATGCCGGC  
10681 ATGGAGAACG ACGCCTCGGT GGCCGAGGAG GATCGAGTCT GGGTGC GCGG CTGGTTTCCG  
10741 ATGCTATTCT CGCTTTCCTG CGTGGTCAAT CGCTGCAAAT TGGATGTGCG TACTCGCGCC  
10801 TTAACCGTGC TTTTTGAGAT TGTGAAGACG TATGGTGAGA GCTTCAAGCC CCATTGGTGG  
10861 AAGGATCTCT TCAATGTGAT CTTCCGTATC TTCGACAACA TGAAATTGCC GGAGCACGTC  
10921 ACCGAGAAGT CCGAATGGAT GACGACCACA TGCAACCACG CCTTGTACGC TATTATTGAT  
10981 GTCTTACGC AGTATTTGCA GTTTCTTGGT CATCTGCTGC TGGAGGAGCT CTTGCCCCAG  
11041 CTGCATTGGT GTGTTTCAGCA GAGTAACGAG CAGTTGGCGC GATCTGGCAC CAATTGCCTG  
11101 GAGAACCTCG TCATTTGCAA TGGATTCAAG TTCAACGAGT CCACCTGGGA CAAGACGTGC  
11161 CAGTGCATCC TGGACATCTT CAACGCCACT TTGCCGAGG ATCTCCTCAG TTGGCGGCCG  
11221 AAAGCACATT CCAGTAACAA TATACCCAG GAGACAACC ACTTTGAGGC GCTGCATATC  
11281 CGTGCGTAG TCCAGCTGGA ACTGATACAG ACCATGGATA ACATTGTCTT TTTCCCGGCC  
11341 ACGTGCAGCA AGGAGGATGC CGAAACGCTG GCCCAGGCGG CGGCAGACTT AACAGGCGGC  
11401 AGGAGCGGTT CGCAGTCGCA GCTGCTGGAG TGCCAGCGGG AGGAGCAGGG AATGTACGGC  
11461 TATCTGAGAA CCCGCCAGCT GCTCACCTG GCCGACTGTC TGATGCAGTC GCACCGTTTT  
11521 GCCAAGCGCT TCAACGCCGA TCACGACCAA CGCAGCCTGC TTTGGCGGGG GGGATTCAAG  
11581 GGATCTGTTA AACCGAATCT GCTGAAGCAG GAGACCTCGT CGCTGGCCTG CGTCTGCGC  
11641 ATTTTCTTCA AGATGTACGG CGACGAGAAT AGACGAGCG ATTGGCCCGG CATCGAGCAG  
11701 GAACTGGTGC AGGTCTGCAA GGAGGCACTG GGCTACTATT TGAGTTTGCA GAGCGAGGCA

```

11761 CACCGAGATG CGTGGACATC GCTGCTGCTG CTCATCCTGA CGCGCCTGCT CAAGATGTCC
11821 GATGCCAGGT TCGCCACCCA CGTTTCCAAC TACTACAGCC TGCTGTGCGA GATGATGTGC
11881 TTCGACCTCA AGCCCGAACT GAGAAGTGTC CTTAGGCGTG TGTTCATGCG CATCGGTCCA
11941 GTATTCAATA TAATGAGCGT TAAATAAttc tagtcgacca tgaagatcaa gatcattgcc
12001 ccgccagagc gcaagtactc tgtctggatc ggtggctcca tcctggcttc gctgtccacc
12061 ttccagcaga tgtggatctc caagcaggag tacgacgagt ccggcccctc catttgtcac
12121 cgcaagtgct tctaagaagg atcgcttgtc tgggcaagag gatcaggatc gggatggtct
12181 tgattctgct ggaggaggag gaggagaagt cgaggaagca gcagcgaaag tgcaagtgcg
12241 agtggtgga gtttggagtg cagcacaaca aaatcaaca caacaccaac tacaagatga
12301 aaagagcgga accacctgca caccatcatc actatcatca tcgttttggg cgcatgttgt
12361 gtggttcag cgtattaata taattaatth attccacatg agatatgata tgatatacta
12421 tgtatttttt gttttttttt tatttgtaa cctttaatat aacaagaact acaaaaaatg
12481 aaaatgagcg aaaatgcata ttctgccatt ccacacacac accaacaaca cccaacacac
12541 gcacacccac aagcttacac acacacattc gcggcatgac aaggacatca agataaagaa
12601 gaacttaaag aagatatttc ccaagcgca aaaagaacac acacacattg caaaacacaa
12661 acaacacact agcgttttgt acaattcgtc agcaacctta tgtattattt ttttaattatg
12721 atgtaattat aaacaaagtg aaacaaaaat atgaaaacaa aaaggaaaat caaatctgtc
12781 ttctctttct cccgctctcc tcgctctctg ctgctaacct cgccctctcc tctctcatct
12841 ttttgtctgt ctctcttcca catttttgcc ggccggcaaa ataataacc acacacactc
12901 acacttggtc gcagtttcgc gtgcgatatt cacacacatt caagcataca agcatacata
12961 catatgtatt ttttttttat ttgtacactt ttctaattgc atgcgatcgc attgataagt
13021 ttacgcctga aatgttaat taaaatgtga aatgcaact gaaaaactga tgaaatgaaa
13081 caacaacaag cgaacaatth gctacatgtg tattgtctaa caaccgttac tgcaacggtt
13141 gcttcggaag aggggtgaaga ggaagagggc acgaggtcga ctagagcggc cgccgacgcg
13201 aggctggatg gccttcccca ttatgattct tctcgcttcc ggcggcatcg ggatg

```

//

**pMK-V5::garz<sup>E740K</sup>**

LOCUS	pMK_V5_garz_E740K	14245 bp ds-DNA	circular	27-FEB-2020
COMMENT	pMK33-CFH-BD from 1 to 8550			
COMMENT	>pMK33-CFH-BD, 8550 bases.			
COMMENT	ApEinfo:methylated:1			
FEATURES	Location/Qualifiers			
CDS	7009..8862			
	/label=garz-PB			
	/ApEinfo_fwdcolor=#99ccff			

```

/ApEinfo_revcolor=#cde7f7
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1} {} 0}
width 5 offset 0
CDS      8908..9273
/label=New Feature
/ApEinfo_fwdcolor=#99ccff
/ApEinfo_revcolor=#99ccff
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1} {} 0}
width 5 offset 0
misc_feature 6493..6860
/label=MT-promoter
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1} {} 0}
width 5 offset 0
misc_feature 4326..5351
/label=Hyg
/ApEinfo_fwdcolor=#ffffcc
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1} {} 0}
width 5 offset 0
misc_feature 6979..6984
/label=SpeI
/ApEinfo_fwdcolor=#ccff66
/ApEinfo_revcolor=#ffffcc
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1} {} 0}
width 5 offset 0
misc_feature 6928..6928
/label=BamHI
/ApEinfo_fwdcolor=#ffcc66
/ApEinfo_revcolor=#ffcc66
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1} {} 0}
width 5 offset 0
primer_bind 11071..11088
/label=garz-GF9
/ApEinfo_fwdcolor=#ff00bd
```

```

    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    6896..6913
    /label=Metallothionein_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
CDS            11089..12954
    /label=garz-PB(1)
    /ApEinfo_label=garz-PB
    /ApEinfo_fwdcolor=#99ccff
    /ApEinfo_revcolor=#cde7f7
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    10513..10532
    /label=garz-GF8
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
rep_origin     complement(777..1459)
    /label=ColE1 origin
    /ApEinfo_fwdcolor=gray50
    /ApEinfo_revcolor=gray50
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    9325..9327
    /label=190Y
    /ApEinfo_fwdcolor=#ccff66
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
CDS            complement(1557..2216)
    /label=AmpR
```

```
    /ApEinfo_fwdcolor=yellow
    /ApEinfo_revcolor=yellow
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    8863..8883
    /label=garz-GF5
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
CDS            7555..7581
    /label=New Feature(1)
    /ApEinfo_label=New Feature
    /ApEinfo_fwdcolor=#99ccff
    /ApEinfo_revcolor=#99ccff
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    9414..9431
    /label=garz-GF6
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
polyA_signal   complement(6293..6484)
    /label=SV40 late polyA
    /ApEinfo_fwdcolor=pink
    /ApEinfo_revcolor=pink
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    7555..7581
    /label=JF01603-F
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature   9490..9709
```



```
    /label=Phosphatidylinositol-phosphate binding
    /ApEinfo_fwdcolor=#ff81f0
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    5645..5664
    /label=pUAST-R
    /ApEinfo_fwdcolor=#0a00ff
    /ApEinfo_revcolor=#0a00ff
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    9916..9935
    /label=garz-GF7
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    13134..13153
    /label=Act5C-qF1
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature    10941..10977
    /label=Phosphatidylinositol-phosphate binding(1)
    /ApEinfo_label=Phosphatidylinositol-phosphate binding
    /ApEinfo_fwdcolor=#ff81f0
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    complement(13341..13360)
    /label=Act5C-qR1
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
```

```
CDS          9710..10940
              /label=Phosphatidylinositol-phosphate binding(2)
              /ApEinfo_label=Phosphatidylinositol-phosphate binding
              /ApEinfo_fwdcolor=#ff81f0
              /ApEinfo_revcolor=#99ccff
              /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
              width 5 offset 0
primer_bind   13068..13087
              /label=Act5C-qF2
              /ApEinfo_fwdcolor=#ff00bd
              /ApEinfo_revcolor=#ff0003
              /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
              width 5 offset 0
primer_bind   7115..7134
              /label=garz-GF3
              /ApEinfo_fwdcolor=#ff00bd
              /ApEinfo_revcolor=#ff0003
              /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
              width 5 offset 0
CDS          10978..11070
              /label=garz-PB(2)
              /ApEinfo_label=garz-PB
              /ApEinfo_fwdcolor=#99ccff
              /ApEinfo_revcolor=#cde7f7
              /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
              width 5 offset 0
primer_bind   complement(13330..13349)
              /label=Act5C-qR2
              /ApEinfo_fwdcolor=#ff00bd
              /ApEinfo_revcolor=#ff0003
              /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
              width 5 offset 0
CDS          8863..8883
              /label=New Feature(2)
              /ApEinfo_label=New Feature
              /ApEinfo_fwdcolor=#99ccff
```

```

    /ApEinfo_revcolor=#99ccff
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
CDS      11071..11088
    /label=New Feature(3)
    /ApEinfo_label=New Feature
    /ApEinfo_fwdcolor=#99ccff
    /ApEinfo_revcolor=#99ccff
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
CDS      10941..10977
    /label=New Feature(4)
    /ApEinfo_label=New Feature
    /ApEinfo_fwdcolor=#99ccff
    /ApEinfo_revcolor=#99ccff
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind 2528..2545
    /label=pQE60-F
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind complement(2135..2159)
    /label=Amp-GF
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 10364..10365
    /label=MB05159
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind 11710..11731
```

```

    /label=garz-GF10
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    1677..1700
    /label=Amp-GR1
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature    9274..9482
    /label=Sec7 domain
    /ApEinfo_fwdcolor=#0080ff
    /ApEinfo_revcolor=#2098da
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    6836..6853
    /label=SEQ-MT-F2
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature    9328..9330
    /label=191A
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    complement(13037..13055)
    /label=Seq-MK-R
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature    9483..9489
```

		<pre>/label=Sec7 domain(1) /ApEinfo_label=Sec7 domain /ApEinfo_fwdcolor=#0080ff /ApEinfo_revcolor=#00ff00 /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0} width 5 offset 0</pre>
CDS	8908..9273	<pre>/label=Sec7 domain EQLAKVKQKKRLLSQGTERFNQRPEKGIQYLQEHGILNAELD /ApEinfo_fwdcolor=#0080ff /ApEinfo_revcolor=#99ccff /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0} width 5 offset 0</pre>
misc_feature	6985..7008	<pre>/label=Linker GL3 /ApEinfo_fwdcolor=#ffcc66 /ApEinfo_revcolor=#ffcc66 /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0} width 5 offset 0</pre>
CDS	8884..8907	<pre>/label=garz-PB(3) /ApEinfo_label=garz-PB /ApEinfo_fwdcolor=#99ccff /ApEinfo_revcolor=#cde7f7 /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0} width 5 offset 0</pre>
primer_bind	7726..7744	<pre>/label=garz-GF12 /ApEinfo_fwdcolor=#ff00bd /ApEinfo_revcolor=#ff0003 /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0} width 5 offset 0</pre>
primer_bind	12328..12346	<pre>/label=garz-GF11 /ApEinfo_fwdcolor=#ff00bd /ApEinfo_revcolor=#ff0003</pre>

	<pre>/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0} width 5 offset 0</pre>
CDS	<pre>9490..9709 /label=New Feature(5) /ApEinfo_label=New Feature /ApEinfo_fwdcolor=#99ccff /ApEinfo_revcolor=#99ccff /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0} width 5 offset 0</pre>
primer_bind	<pre>9729..9757 /label=garz-7193-F /ApEinfo_fwdcolor=#ff00bd /ApEinfo_revcolor=#ff0003 /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0} width 5 offset 0</pre>
misc_feature	<pre>6937..6978 /label=V5 /ApEinfo_fwdcolor=#ffffcc /ApEinfo_revcolor=#ffffcc /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0} width 5 offset 0</pre>
CDS	<pre>9483..9489 /label=New Feature(6) /ApEinfo_label=New Feature /ApEinfo_fwdcolor=#99ccff /ApEinfo_revcolor=#99ccff /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0} width 5 offset 0</pre>
primer_bind	<pre>complement(10211..10236) /label=garz-7193-R /ApEinfo_fwdcolor=#ff00bd /ApEinfo_revcolor=#ff0003 /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0} width 5 offset 0</pre>
misc_feature	<pre>9223..9225 /label=E740K</pre>



```

    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    8000..8022
    /label=Rab1 binding
    /ApEinfo_fwdcolor=#66ccff
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    complement(8000..8022)
    /label=garz-JF01603-R
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    7923..7923
    /label=New Feature(7)
    /ApEinfo_label=New Feature
    /ApEinfo_fwdcolor=#ffffcc
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    7931..7931
    /label=New Feature(8)
    /ApEinfo_label=New Feature
    /ApEinfo_fwdcolor=#ffffcc
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    8238..8256
    /label=garz-GF4
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
```

ORIGIN

1 cccgcgttgc aggccatgct gtccaggcag gtagatgacg accatcaggg acagcttcaa  
61 ggatcgctcg cggctcttac cagcctaact tcgatcattg gaccgctgat cgtcacggcg  
121 atttatgccg cctcggcgag cacatggaac gggttggcat ggattgtagg cgccgcccta  
181 taccttgtct gcctccccgc gttgcgtcgc ggtgcatgga gccgggccac ctcgacctga  
241 atggaagccg gcggcacctc gctaacggat tcaccactcc aagaattgga gccaatcaat  
301 tcttgcgag aactgtgaat gcgcaaacca acccttgga gaacatatcc atcgcgtccg  
361 ccatctccag cagccgcacg cggcgcactc cgggcagcgt tgggtcctgg ccacgggtgc  
421 gcatgatcgt gctcctgtcg ttgaggacc ggctaggctg gcggggttgc cttactggtt  
481 agcagaatga atcaccgata cgcgagcga cgtgaagcga ctgctgctgc aaaacgtctg  
541 cgacctgagc aacaacatga atggtcttcg gtttccgtgt ttcgtaaagt ctgaaacgc  
601 ggaagtcagc gctcttccgc ttctcgtc actgactcgc tgcgctcggc cgttcggctg  
661 cggcgagcgg tatcagctca ctcaaaggcg gtaatacggc tatccacaga atcaggggat  
721 aacgcaggaa agaacatgtg agcaaaaggc cagcaaaagg ccaggaaccg taaaaaggcc  
781 gcgttgctgg cgtttttcca taggctccgc cccctgacg agcatcaca aaatcgacgc  
841 tcaagtcaga ggtggcgaaa cccgacagga ctataaagat accaggcgtt tccccctgga  
901 agctccctcg tgcgctctcc tgttccgacc ctgccctta ccggatacct gtccgccttt  
961 ctcccttcgg gaagcgtggc gctttctcat agctcacgct gtaggtatct cagttcggtg  
1021 taggtcgttc gctccaagct gggctgtgtg cacgaacccc ccgttcagcc cgaccgctgc  
1081 gccttatccg gtaactatcg tcttgagtc aaccggtaa gacacgactt atcgccactg  
1141 gcagcagcca ctggtaacag gattagcaga gcgaggtatg taggcggtgc tacagagttc  
1201 ttgaagtggc ggcctaacta cggctacact agaaggacag tatttggtat ctgcgctctg  
1261 ctgaagccag ttaccttcgg aaaaagagtt ggtagctctt gatccggcaa acaaaccacc  
1321 gctggtagcg gtggtttttt tgtttgcaag cagcagatta cgcgcagaaa aaaaggatct  
1381 caagaagatc ctttgatctt ttctacgggg tctgacgctc agtggaacga aaactcacgt  
1441 taagggatth tggatcatgag attatcaaaa aggatcttca cctagatcct tttaaattaa  
1501 aatgaagtt ttaaatcaat ctaaagtata tatgagtaaa cttggtctga cagttaccaa  
1561 tgcttaatca gtgaggcacc tatctcagcg atctgtctat ttcgttcacc catagttgcc  
1621 tgactccccg tcgtgtagat aactacgata cgggagggtc taccatctgg cccagtgct  
1681 gcaatgatac cgcgagacc acgctcaccg gctccagatt tatcagcaat aaaccagcca  
1741 gccggaaggg ccgagcgag aagtggctct gcaactttat ccgcctccat ccagtctatt  
1801 aattggtgcc ggaagctag agtaagtagt tcgccagtta atagtttgcg caacgttgtt  
1861 gccattgctg caggcatcgt ggtgtcacgc tcgtcgttt gtaggtctc attcagctcc  
1921 ggttcccaac gatcaaggcg agttacatga tccccatgt tgtgcaaaaa agcggtagc  
1981 tccttcggtc ctccgatcgt tgtcagaagt aagttggccg cagtgttatc actcatggtt  
2041 atggcagcac tgcataattc tcttactgtc atgccatccg taagatgctt ttctgtgact

2101 ggtgagtact caaccaagtc attctgagaa tagtgtatgc ggcgaccgag ttgctcctgc  
2161 ccggcgtcaa cacgggataa taccgcgcca catagcagaa ctttaaaagt gctcatcatt  
2221 ggaaaacgtt cttcggggcg aaaactctca aggatcttac cgctgttgag atccagttcg  
2281 atgtaacca ctcgtgcacc caactgatct tcagcatcct ttactttcac cagcgtttct  
2341 gggtgagcaa aaacaggaag gcaaaatgcc gcaaaaaagg gaataagggc gacacggaaa  
2401 tgttgaatac tcatactcct cttttttcaa tattattgaa gcatttatca gggttattgt  
2461 ctcatgagcg gatacatatt tgaatgtatt tagaaaaata acaaatagg ggttccgcgc  
2521 acatttcccc gaaaagtgcc acctgacgtc taagaaacca ttattatcat gacattaacc  
2581 tataaaaaata ggcgtatcac gaggcccttt cgtcttcaag aattcacatt tgtacgaatt  
2641 ttttttttat caaaagttcg agtttttcac caatttcctc atcaaccgag caaggcaaac  
2701 ggctttgaat aatatggtgt tatatataca tatatcaaat cgctgctgac tgcgtgattg  
2761 atggccccaa gattacatat tatcgaatca ggattcagaa ggagatcaat gtcaaatgcg  
2821 gacaggaaca tgaagacgc ctgttatgcy caattaaaaa tttgggttta attgctgtgg  
2881 aaactgttgt tggcggcatc ttaagttcct gtttaacaac atcaactact tatgtacgta  
2941 gaagcgttta agccatttgc atacagatga gaactggctt ttgtgctaata cagtcaagat  
3001 gactccgatg atgatgactc attacctgac cagttttcgc tgctttcctt tcaacaacta  
3061 cttgtatatg tattgtatcc aatagcaata cattgaattt ccatggctca gtcacgtatt  
3121 atcatttaat tgacaccaag tcgtgttatt gttgagctat cgagttcagc tcaaacattt  
3181 cttattccca tgaataagcc ggcaaaaaata tgcaatctat gaaagttaat ataagcaaac  
3241 cttactttga ctcaatacca atgcactttg tgcgataggg ttcacgcaat tgaggcgatt  
3301 attccgataa cccaagcgat tgactgttcc cgtttcgatt ccaattgaaa tttggaaatg  
3361 tacaatagtt ttgctatatg ctgtcaagta cgctcttacc ttctctgggt tttcttcaga  
3421 gtttcgaaac gcttcttctt ttttttgttt tttttttttt ggaatctcgt attttggaaag  
3481 gggctcccct ctggaatttg ttacactgtc gttatcattg cgaacaagcg gcccgaaact  
3541 atcagcgact ttaacattta caatgcactt ttttacgacc aattaaatgt acattttcct  
3601 ttcttcgccc gttgataagc gaacgcgatg tggcgcaggc aatgtgttgc tcttgcgaca  
3661 caaacgcaat caaatggat tcaatttcgc tttttccag tgaacgaag aacgaaccga  
3721 ccatcatgat atgctcctct gcatgttgcg tattgaaatca atgacaattt caattaagcc  
3781 gcccgttcgt catgcgtttt cgtgcgcttc gaaatgctga taacgctgct gtcctccaac  
3841 tgctttgcat gtggacacaa ttccatttat ttaattcttt tatttggatc ggtaaatta  
3901 aaaagcgcct tgttacgcat ttaacgttgt ttccgggtgcg tgggtggttc atgcttctgg  
3961 gaacggcaaa tgggttttagg attgggaacc cctcatcacc tgttggaata tactattcaa  
4021 cctacaaaag taacgttaaa caaactact ttatatttga tatgaaatgg cacacctttt  
4081 atgccataaa acatatttga agagaatacc actcttttta ttccttcttt ctttcttga  
4141 cgttttttgc tgtaagtagg tcgtggtgct ggtgttgcaag ttgaaataac ttaaaatata  
4201 aatcataaaa ctcaaacata aacttgacta tttatttatt tattaagaaa ggaaatataa

```
4261 attataaatt acaacaggtt atggacctgc agccaagctt ggcgctcgtc cgggggcaat
4321 gagatATGAA AAAGCCTGAA CTCACCGCGA CGTCTGTCTGA GAAGTTTCTG ATCGAAAAGT
4381 TCGACAGCGT CTCCGACCTG ATGCAGCTCT CGGAGGGCGA AGAATCTCGT GCTTTCAGCT
4441 TCGATGTAGG AGGGCGTGGA TATGTCCTGC GGGTAAATAG CTGCGCCGAT GGTTTCTACA
4501 AAGATCGTTA TGTTTATCGG CACTTTGCAT CGGCCGCGCT CCCGATTCCG GAAGTGCTTG
4561 ACATTGGGGA ATTCAGCGAG AGCCTGACCT ATTGCATCTC CCGCCGTGCA CAGGGTGTCA
4621 CGTTGCAAGA CCTGCCTGAA ACCGAACTGC CCGCTGTTCT GCAGCCGTC GCGGAGGCCA
4681 TGGATGCGAT CGCTGCGGCC GATCTTAGCC AGACGAGCGG GTTCGGCCCA TTCGGACCGC
4741 AAGGAATCGG TCAATACACT ACATGGCGTG ATTTTATATG CGCGATTGCT GATCCCCATG
4801 TGTATCACTG GCAAACCTGT ATGGACGACA CCGTCAGTGC GTCCGTCGCG CAGGCTCTCG
4861 ATGAGCTGAT GCTTTGGGCC GAGGACTGCC CCGAAGTCCG GCACCTCGTG CACGCGGATT
4921 TCGGCTCCAA CAATGTCCTG ACGGACAATG GCCGCATAAC AGCGGTCATT GACTGGAGCG
4981 AGGCGATGTT CGGGGATTCC CAATACGAGG TCGCCAACAT CTTCTTCTGG AGGCCGTGGT
5041 TGGCTTGAT GGAGCAGCAG ACGCGCTACT TCGAGCGGAG GCATCCGGAG CTTGCAGGAT
5101 CGCCGCGGCT CCGGGCGTAT ATGCTCCGCA TTGGTCTTGA CCAACTCTAT CAGAGCTTGG
5161 TTGACGGCAA TTTCGATGAT GCAGCTTGGG CGCAGGGTCG ATGCGACGCA ATCGTCCGAT
5221 CCGGAGCCGG GACTGTCGGG CGTACACAAA TCGCCCGCAG AAGCGCGGCC GTCTGGACCG
5281 ATGGCTGTGT AGAAGTACTC GCCGATAGTG GAAACCGACG CCCCAGCACT CGTCCGAGGG
5341 CAAAGGAATA Gagtagatgc cgaccgaaca agagctgatt tcgagaacgc ctcagccagc
5401 aactcgcgcg agcctagcaa ggcaaatgcy agagaacggc cttacgcttg gtggcacagt
5461 tctcgtccac agttcgctaa gctcgtcgg ctgggtcgcg ggagggccgg tcgcagtgat
5521 tcaggccctt ctggattgtg ttgggtccca gggcacgatt gtcattgcca cgcactcggg
5581 tgatctgact gatcccgcag attggagatc gccgcccgtg cctgccgatt ggggtgcagat
5641 ctttgtgaag gaaccttact tctgtggtgt gacataattg gacaaactac ctacagagat
5701 ttaaagctct aaggtaaata taaaatthtt aagtgtataa tgtgttaaac tactgattct
5761 aattgtttgt gtatthttaga ttccaaccta tggaaactgat gaatgggagc agtgggtggaa
5821 tgcctthaat gaggaaaacc tgthttgctc agaagaaatg ccatctagtg atgatgaggc
5881 tactgctgac tctcaacatt ctactcctcc aaaaaagaag agaaaggtag aagaccccaa
5941 ggactthcct tcagaattgc taagthtttt gagtcatgct gtgthttagta atagaactct
6001 tgcttgctth gctatthtaca ccacaaagga aaaagctgca ctgctataca agaaaattat
6061 ggaaaaatat tctgtaacct ttataagtag gcataacagt tataatcata acatactgtt
6121 thttcttact ccacacaggc atagagtgtc tgctatthaat aactatgctc aaaaattgtg
6181 tacctthtagc thtttaatth gtaaaggggt taataaggaa thtttgatgt atagtgcctt
6241 gactagagat cataatcagc cataccacat ttgtagagggt thtacttgct thaaaaaac
6301 tcccacacct cccctgaac ctgaaacata aatgaaatgc aattgttgtt gthaaactgt
6361 thattgcagc thataatggt tacaataaa gcaatagcat cacaaatthc acaataaag
```

```
6421 catttttttc actgcattct agttgtggtt tgtccaaact catcaatgta tcttatcatg
6481 tctggatcaa ttCGTTGCAG GACAGGATGT GGTGCCCGAT GTGACTAGCT CTTTGCTGCA
6541 GGCCGTCCTA TCCTCTGGTT CCGATAAGAG ACCCAGAACT CCGGCCCCC ACCGCCACC
6601 GCCACCCCA TACATATGTG GTACGCAAGT AAGAGTGCCT GCGCATGCC CATGTGCCC
6661 ACCAAGAGCT TTGCATCCCA TACAAGTCCC CAAAGTGGAG AACCGAACCA ATTCTTCGCG
6721 GGCAGAACAA AAGCTTCTGC ACACGTCTCC ACTCGAATTT GGAGCCGGCC GGCCTGTGCA
6781 AAAGAGGTGA ATCGAACGAA AGACCCGTGT GTAAAGCCGC GTTTCCAAAA TGTATAAAAC
6841 CGAGAGCATC TGGCCAATGT gcatcagttg tggtcagcag caaaatcaag tgaatCATCT
6901 CAGTGCAACT AAAGGGGGGA TCTcgagGCC ACCATGGGTA AGCCTATCCC TAACCCTCTC
6961 CTCGGTCTCG ATTCTACGAC TAGTGGAGGA GGAGGTTCTG GTGGTGGTGC GCTTCCAGGC
7021 AACGGCATCT ACGTGGTGGC GGGCGAAATG GCCACCCTGA TGACGGCGAT GCGACGTGGA
7081 ACGCGTTGGA ATGCCACCGC CTACGTGGAC GACGAGAATG ACTCGCTGCT GAAGCTGTTC
7141 ATTGACCTCA AGCATGAGCT AAATCGGATC GAGGACCTGC GTCAGATCGA GCCTCAGGTC
7201 TTCCTGGCTC CGTTTCTTGA GGTGATTCGC ACGGCGGACG CCACGGGTCC GTTGACTAGT
7261 CTAGCCTTGG CCTCGTTAA CAAATTATTG TCTTACGGC TAATAGATCC CACGTCTCCA
7321 AATCTGGCTG ACATTGTGGA GCGCATTGCC GATGCTGTGA CACATGCCG CTTTATGGGC
7381 ACCGATCAGT CCTCGGATGG TGTCACCTTT ATGCGAGTGA TTGAGGTGCT GCACACGCTT
7441 ATCCGAGTC CCGAAGGAGC CGCCGTCAGC AATGTGTCCA TGTGCGAGGT GATGCTCAGC
7501 TGCTTCAAGA TCTCCTTGA GCCGAGGCTG AGTGAAGTGC TTCGTCGCTC GCGGAAAAA
7561 TCGCTCAAGG ACATGGTCTG GCTCTTCTC ATGCGCCTC CCCAGTTCGC CGAAGAGCGA
7621 AGTGACACCA TGCTCCAGAA GCGATTTACT ATCGGCGATG CTGCCAGCGG AGCTACCCAA
7681 GAAAAACTAA AGCGTAAGAC GGTTGCCCAA GCCCAGACAG CACCCAGGAA ATCGTCAGCA
7741 GTGGAGGAAC CGCCTCAAAC ACCGCAGTCT GCCAACCTGA CGGTGCCAGG GCACTTGAAG
7801 GCACCCATAT TGGCCACCAC ACCCGCCAGT CCAGCGGGAA ACATATTGGA CATGCAGGGC
7861 AAGATTACGC AGACACCAAC AACACGGCG AGCACGGGG AAGATGAAAC CACTGTCCCG
7921 GAGACTCCTG TTATTCAAGT GGAGTCGACT GAATCGGAGC CTTGCTGGA CGGCGAAACG
7981 GGTGAAGCAA CCAGCACCTT GGCCGAGGCA AATAGTAGCG AGTACATCAA CTCGGTGGGC
8041 GTCCGTTTTA CACAGCAGTC CACCGATCAC GATGTAACAT CACTATCCCC TTATGGCCTG
8101 CCCTTCATCC AGGAGTTGTT CCGATTCTC ATAATCCTCT GCAATCCTCT GGATAAGCAG
8161 AACTCGGATA GCATGATGCA CACGGTCTT AGTCTACTTA CTGTAGCTTT TGAAGTCGCA
8221 GCCGATAATA TTGAAAAGTA TGAGGGCTTG CTGGAGCTGG TTAAGGACGA CTTATGCAGA
8281 AACTTAATAT CGCTTCTCAG CTCAGAGCGG CTTAGCATCT TTGCCGCCGA TTTGCAGCTC
8341 TGTTTTTTGC TTTTCGAGTC TCTCCGCGGA CATCTCAAGT TTCAGCTGGA AGCCTACCTT
8401 AGAAAATTGA GCGAGATTAT TGCTAGCGAT AATCCCAAGA CGCCCTACGA AATGCGAGAA
8461 CTCGCTCTGG ACAATCTACT GCAGTTGTGG CGCATTCCCG GCTTCGTAC GGAATTGTAT
8521 ATCAACTACG ATTGTGACTT GACTGCACG GATATGTTT AAAGTTTGAC AAACCTACTG
```

8581 AGCAAGTATA CGCTGTCAGC AACGAATGCA GTTTATAGCA CCCACATTAT CTCAATGGAC  
8641 ACCCTGTAA GTGTGATAGA CAGTATCGAG CGAAATTGTG CCGCGAGCAA GAATAGCAGC  
8701 AACAAACAGAG AGTCCTTGCC AGAAGCTGCC CCAGCAACAG GTGGCAGCCG CCATTCTCGC  
8761 CACAACAGCG GATTGGAGGG AATCGTAATT GATTCTGGCA ATAGTGTAGC TGCAGAAGAG  
8821 AAAGTGGAGA ACATCGCAAG CTTTATAAAT GCGAGCTCAC ATCGACTACG ACTACAATCT  
8881 GCGGAGAGG GAGTGGGAAT AACCAGTGAA CAGCTGGCCA AGGTCAAACA GAAGAAGCGT  
8941 CTGCTATCCC AAGGCACAGA GCGATTAAAT CAGCGTCCAG AGAAAGGAAT CCAGTATCTG  
9001 CAAGAACACG GCATCCTAAA TGCCGAGCTT GATCCCATGC AGGTGGCCCT GTTCCTTCGA  
9061 GAAAATCCCG GGCTCGATAA AAAAATGATT GCGGAATATA TCTCGAAAAA GAAAAACGTC  
9121 GACTCTAAGA TTCTAATTAA TTTTGTGGAC TCGTTTGATT TCACTGGTCT TCGAGTGGAT  
9181 CAAGCATTGC GTCTTTATCT GGAGACCTTC AGATTGCCCG GAAGGCTCC ATTGATCTTT  
9241 TTGGTGCTGG AACACTTTTC TGATCATTGG CATAAACAAA ACCAAGATCC GTTGGCCAAC  
9301 GTAGACGCTG CTTTTGCTT GGCCTATGCC ATCATCATGC TGAACATGGA TCAGCACAAAC  
9361 TCGAACGCGA AGCGTTTAAA TGTTCCAATG ACGCTCGAGG ACTTACTAA GAATTTGCGT  
9421 GGTCTAAACG GTGGCGAAGA TTTTCGATCAA GAAATGCTGG CTCAAGTCTT TAATGCAATC  
9481 AAGAACGAAG AGATCGTTAT GCCAGCAGAG CAAACGGGTC TGGTGCCTGA AAATTATCAA  
9541 TGGAAAGTAC TGCTTCGACG AGGAGACACG CACGATGGAC ATTTTACTA TGTGCATGAC  
9601 GCATCATACG ACGTGGAGAT CTTCAATATT GTGTGGGGTG CTTCTCTGAG CGCCCTAAGC  
9661 TTTATGTTTG ATAAAAGCAC TGAAACGGGC TACCAAAGAA CTCTAGCAGG TTTCAGCAAA  
9721 TCCGCTGCCA TATCGGCGCA CTATAATCTG CATTCCGACT TCGATGCCCT CGTTTTAACT  
9781 CTCTGCAAAT TCACAACGCT GCTGAGCAGC GTAGAACAGC ATGAGCCCGC TCCGGCGAAC  
9841 AATGAAACCC AGCAAGCTGT GAACTTTGGA TTGAACGGAA AGGCTCAGGC TGCCATGCGA  
9901 ACGGTGTTTC TATTGTTCA CGACTACGGC GATTGCTTAA GAGAGAGCTG GAAACACATT  
9961 TTGACCTAT ATCTGCAGCT TTTCCGTCTA AAGTTGCTGC CAAAATCATT GATCGAAGTG  
10021 GAAGACTTTT GTGAGGCGAA CGGAAAGGCC ATGTTAATCC TGGAAAAGCC CCGCGAGAAG  
10081 CAGGAATCGG GACTATTTTC CAGCCTGTAC TCATTTATCA GCTCGAGGG TCAGCGAGAA  
10141 CCAACGTACG AGGAGCAGGA CTTTCATCAA CTGGGACGGA AGTGCATTAA GGAGTGCCAG  
10201 CTGGATCAA TGCTGCAGGA ATCAAAGTTT GTGCAACTAG AGTCGCTGCA GGAGTTGCTT  
10261 AAATGCGTTC TAGCGCTACT GAAGGCTCCT CAGGGGCACA AATCCATTGG CCTGCCGTAC  
10321 GCCGAAGATC AAAGTGTTC CTGGATGGAA TTTTGGTCA AGATAGTTGT TCATAACCGG  
10381 GATCGCATGA TACCGCTGTG GCCAGCAGTT CGAGACCAA TGTACCTACT GCTTATGGGC  
10441 AGTGCCTCCT GTGGATACGA CTACCTACT AACCAGTGA TTGTAGCGGT CCTAAAATA  
10501 GCTATCTATC TGATGCGAAA CGAAGAAGTGT TGTCCGATCG TATTGCAATC GCTCAAGATG  
10561 CTTTTAATGC TTAAGCCAGC CTTGTTGCTG CGCATTCTA AACAGATTTC CATTGGTATC  
10621 TATGAGCTGC TCAAGACGTC GGCCAAAAT ATTCATTCCG AGCAGGACTG GCAGATTATT  
10681 TTCAATCTAC TTGAATGCGT GGGAGCCGGT GCTGTGCCG CCAATTATGA TGATGCCAG



10741 CTGCCATTGC CGCCCAACGG AAGTGCAAAG TCTGATGGCG CTATAAGTGG CGAAGAGGAC  
10801 GCAACTGCCG TGCCAGAGCG TGGTTACACT TCGGATTTCG AGATCACGAA AGCATCTGCA  
10861 GCACCTGCAG TCTCCAGTCC AAGTGCTGAG AACTGGATTG TGGTCAATAA CAAGGACAGT  
10921 GAATTGACTA CGGCCTCTAG ACCACAATCT CCGCCTAGCC TGAGTGCTCC TCCAGTAAAT  
10981 ACGCTTGTGT ACAATTGCCA GCTACTAGAC CACGCTCCGT TTGCTCTTTT CAAGTGCTGG  
11041 GATTTCGCTGG CGTTTATCGT GCGCAGTGTG GCACACATCA CGCCTTACAA TTTTGAAGCC  
11101 TGC GTTCGCT GCATCCGCAT CTTTGTGGAG GCTTGTGGG ATGGAGGTAT ACGCCAGCGG  
11161 CGAAAGCTGG AATCGGCGGC TAAGCAGAAA AGTTCCAAGA AGCGCAGCGA ACGCAAACCG  
11221 GGCATGGCTT CCTCCGCCTC GAGTAGTAAT CTTACTCTTC TGACGGGCGA CCCGTCCGAC  
11281 AACAGATAA ACGGAAATGC GGCAGAGCAG GAGGACCTGG CCCAGCGCTA CGAACAGTTG  
11341 TCCATTCAAC TGCTGGACCT GATGTATACG TTGTACACGC GAACTGCCCA AATCTTCCGA  
11401 TGGTGGGCGG AAGAAGGATG CACAGTGCCG CAGTCGGCAG CTTTGTGGTC ACCGGGCTGG  
11461 TGTCATTGC TTCAGGGAAT CGCCAGGCTG GCAATGGATC GACGGCGAGA GGTGCGCACC  
11521 CATGCCATAT CGTGCCTGCA GCAGCGGGCA TTGCTAGTCC ATGACCTGCA AACGTTGTGC  
11581 GGAACGGAGT GGTGCTCTTG CTTCCACCAG GTGCTGTTC CCCTCCTAAA CGAACTGCTG  
11641 CCCGAGAGTA ATGCAGCCGG CCAACTGGAT GCCGCTCTCC TCGAAGAGTC GCGTATACGA  
11701 ACGGCCACCA TTATGTCTAA GGTGTTCTG CAACACCTGA CCACGCTCAT CGAGCTGGGA  
11761 AATGCTTTTA ACGAGCTGTG GCTGGATATA TTGACTACA TTGAGCGCTT TATGAAGGTG  
11821 GGATCGGACA CATTGTCCGA GCAGATGCAG GAGATACTGA AGAACATGCT GCTGGTGATG  
11881 CATTAGTGC GAGTGTTCCA CAATCAGGAT GGTAGTTTAC AGCAGGCTCT TTGGGAGCTA  
11941 ACCTGGCGAC GCATCGGCGA ATTTTTGCC AACCTGAAGG AGGAGCTTTT CCACGACGAA  
12001 GGCAAGCGAG CTCAGACCTT AACGAACCCA GCTCCACAGG CAGCTGTGGC TGCCGCTCCA  
12061 CAGCAACAGT TACCAGCGGT GACCATTTTG CCCAGGCAAA CCCAGTTTC CAACGAGTTA  
12121 GTGGTGAGCG CGCCTACTCC GCCGCGAGCC ACACCTTTCG TGGGCTCTCC CGTCGAGTCG  
12181 CCGAGGCGGA GCATAATACT GCAGCCACCC ATGGCCGATG TACTGCAACA GCCGCCAGC  
12241 TTTGTATTTG CTCAGCCCAT TATTGTGCCA CCCCAGCCGC CTGCAGTTAC GGACCCAATA  
12301 CCACCAAGTA CATTATTGCC GGATTTGGTG AATGAGGCAA CTGCTGCTGC CGTGCAAGCC  
12361 ACGACCACGT CCCCAGCGCA CAGCCCGCAG GAGGCGGAGC AGCCGGCTTC AATAGTGCAG  
12421 CAGACCAACA TCGTAACCAC CAACAATACG TACAATAGCT ACGCCATTGA GGTGCCCATG  
12481 GCGCCGAGGA CAACTGCGGA ACAGTTTGGG CAGCAGCAGC AGCAACTGCT TTACCAACAG  
12541 TACTATCAAC AGTATCAGGC CCAGCAGCAA CAGTTGCCGG CTCCAGCCAG CGACCCTGCC  
12601 ATCAATGTGC CAATTAGTCA TCTGCTGGCC GGAAATGCGT ACCCCTCGCT CCCAAAATG  
12661 CCGCAGGCAT CCATTGTGCA CAGCTTTGCG CCCGTTTACG AAAGCCAGGC GGCGACGAGT  
12721 GGAGCTGGGA CAGCAGCAGC GGACATCTAT CAGGAGTATG TGCAAAATCC GTACAACCTT  
12781 ACGTTGCAAC AGCATCCCCA ACAGCAGCTC CATCAGCAGC AGCAGCAGCA ACAACAGCAG  
12841 GCTACAGGAA TGGCCAACGC ATTTCCCGCC GTTGCCACGC CAGCCAACCTA CTTTAATGTG

```

12901 AATGTGGACC CCAGTAGCAT ACCGCCCGGA TCGGAAGTGC TCTACGGCCA GCAGTAATtc
12961 tagtcgacca tgaagatcaa gatcattgcc ccgccagagc gcaagtactc tgtctggatc
13021 ggtggctcca tcctggCTTC GCTGTCCACC TTCCAGcaga tgtggatctc caagcaggag
13081 tacgacgagt cggcccctc cattgtgcac cgcaagtgct tctaagaagg atcgcttgtc
13141 tgggcaagag gatcaggatc gggatggtct tgattctgct ggaggaggag gaggagaagt
13201 cgaggaagca gcagcгааag tgcaagtgcg agtggtgгaa gtttgгagtг cagcacaaca
13261 aatcaacaa caacaccaac tacaagatga aaagagcgga accacctgca caccatcatc
13321 actatcatca tcgttttggg cgcattgtgt gtggttcag cgtattaata taattaattt
13381 attccacatg agatatgata tgatatacta tgtatTTTTT gTTTTTTTT tatttgtaa
13441 ctttaatat aacaagaact acaaaaaatg aaaatgagcg aaaatgcata ttctgccatt
13501 ccacacacac accaacaaca cccaacacac gcacaccac aagcttacac acacacattc
13561 gcggcatgac aaggacatca agataaagaa gaacttaaag aagatatttc ccaaagcgca
13621 aaaagaacac acacacattg caaacacaa acaacacact agcgTTTTgt acaattcgtc
13681 agcaacctta tgtatttttt tttaattatg atgtaattat aaacaaagtг aaacaaaaat
13741 atgaaaacaa aaaggaaaat caaatctgtc ttctcttct cccgctctcc tcgctctctg
13801 ctgctaacct cgcctctcc tctctcatct tttgtctgt ctctctcca catttttggc
13861 ggccggcaaa ataataacc acacacact acacttggt gcagtttcgc gtgcgatatt
13921 cacacacatt caagcataca agcatacata catatgtatt tttttttat ttgtacactt
13981 ttctaattgc atgcgtatcg attgataagt ttacgcctga aatgttaat taaaatgtga
14041 aatgcaact gaaaaactga tgaaatgaa caacaacaag cgaacaattt gctacatgtg
14101 tattgtctaa caaccgttac tgcaacggtt gcttcggaa agggтgaaga ggaagaggгc
14161 acgaggтcga ctagagггc cгccgacгcг aggгггatг gccttccca ttatgattct
14221 tctcггtcc ggcггcатcг ggatg

```

//

**pMT-hyg-V5::Sec71**

```

LOCUS      pMT_hyg_V5_Sec71      10266 bp ds-DNA      circular      28-FEB-
2020
DEFINITION pMT-puro Sequencing Result
ORGANISM   other sequences; artificial sequences; vectors.
COMMENT    pMT-vAX2m from 1 to 5573
COMMENT    pMT-vSOG4m from 1 to 5126
COMMENT    pMT-mSOGm_v4 from 1 to 5114
COMMENT    ApEinfo:methylated:1
FEATURES   Location/Qualifiers
            misc_feature   complement(10177..10196)
                        /label=EBV_rev_primer

```

```
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature    complement(10134..10151)
    /label=BGH_rev_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature    10094..10111
    /label=6xHis
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature    10043..10084
    /label=V5
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
CDS            5014..9969
    /label=Sec71
    /ApEinfo_fwdcolor=#99ccff
    /ApEinfo_revcolor=#cde7f7
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature    9567..9567
    /label=T>G silent
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature    10031..10036
    /label=ApaI GGGCC^C
```

```
    /ApEinfo_fwdcolor=#ffffcc
    /ApEinfo_revcolor=#ffffcc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    7770..7789
    /label=sec71-GF6
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    7629..7629
    /label=A>G in 40A
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    4927..4932
    /label=KpnI
    /ApEinfo_fwdcolor=#ffffcc
    /ApEinfo_revcolor=#ffffcc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
protein_bind    6733..7305
    /label=Sec7 domain
    /ApEinfo_fwdcolor=#0080ff
    /ApEinfo_revcolor=#0080ff
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    9552..9552
    /label=C>T silent
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    7250..7273
    /label=Sec71-DRSC01893-F (8A10)
```

```
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    9054..9073
    /label=sec71-GF8
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    8509..8530
    /label=sec71-GF7
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    complement(8562..8583)
    /label=Sec71-GR3
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature   6069..6069
    /label=C>T confirmed in 40A 2013
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature   10011..10030
    /label=New Feature
    /ApEinfo_fwdcolor=#ffffcc
    /ApEinfo_revcolor=#ffffcc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature   7557..7557
    /label=T>C in 40A
```

```

    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    7668..7668
    /label=A>C in 40A
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    9662..9679
    /label=Sec71-GF10
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    7197..7215
    /label=Sec71-GF11
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    6797..6820
    /label=Sec71-GF12
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    7159..7161
    /label=M717(M194 in sec7 domain M>L = BFA-resistant)
    /label=M717L(BFA-resistant)
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    6903..6925
```



```

        /label=Sec71-GF14
        /ApEinfo_fwdcolor=#ff00bd
        /ApEinfo_revcolor=#ff0003
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
misc_feature    complement(7099..7121)
        /label=Sec71-crRNA7(Protospacer)
        /ApEinfo_fwdcolor=#ccff66
        /ApEinfo_revcolor=#ffff9f
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
misc_feature    7188..7207
        /label=Sec71-crRNA8 Protospacer
        /ApEinfo_fwdcolor=#ccff66
        /ApEinfo_revcolor=#ffffcc
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
misc_feature    7828..7828
        /label=T>C Val>Ala in FRT40A
        /ApEinfo_fwdcolor=#ccff66
        /ApEinfo_revcolor=#00ff00
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
CDS            7890..7890
        /label=Sec71-PA
        /ApEinfo_fwdcolor=#99ccff
        /ApEinfo_revcolor=#cde7f7
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
CDS            5977..5979
        /label=Sec71-PA(1)
        /ApEinfo_label=Sec71-PA
        /ApEinfo_fwdcolor=#99ccff
        /ApEinfo_revcolor=#cde7f7
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
```

```
misc_feature    9363..9363
                /label=T>C silent
                /ApEinfo_fwdcolor=cyan
                /ApEinfo_revcolor=#00ff00
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
misc_feature    8509..8530
                /label=Chang-F1
                /ApEinfo_fwdcolor=cyan
                /ApEinfo_revcolor=#00ff00
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
primer_bind     5568..5587
                /label=sec71-GF3
                /ApEinfo_fwdcolor=#ff00bd
                /ApEinfo_revcolor=#ff0003
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
misc_feature    7890..7890
                /label=A>C silent
                /ApEinfo_fwdcolor=cyan
                /ApEinfo_revcolor=#00ff00
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
CDS             6069..6069
                /label=Sec71-PA(2)
                /ApEinfo_label=Sec71-PA
                /ApEinfo_fwdcolor=#99ccff
                /ApEinfo_revcolor=#cde7f7
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
CDS            6600..6600
                /label=Sec71-PA(3)
                /ApEinfo_label=Sec71-PA
                /ApEinfo_fwdcolor=#99ccff
                /ApEinfo_revcolor=#cde7f7
```

```

    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature    9447..9447
    /label=G>A silent
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature    7150..7152
    /label=S191
    /ApEinfo_fwdcolor=#66ff66
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature    9751..9751
    /label=T>C silent(1)
    /ApEinfo_label=T>C silent
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind     6550..6569
    /label=Sec71-GF5
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind     complement(7726..7761)
    /label=Sec71-DRSC01893-R
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature    9324..9324
    /label=T>C silent(2)
    /ApEinfo_label=T>C silent
```

```
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    complement(9224..9245)
    /label=Chang-R1
    /ApEinfo_fwdcolor=#fb53d0
    /ApEinfo_revcolor=#fc5a5d
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    5924..5943
    /label=sec71-GF4
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    7039..7041
    /label=E677 (E740 in garz)
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    6600..6600
    /label=G>A silent(1)
    /ApEinfo_label=G>A silent
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    7147..7149
    /label=F190 F190Y=BFA-hypersensitive
    /label=F713Y(BFA-sensitive)
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
```

```
misc_feature    7719..7719
                /label=A>G 40A
                /ApEinfo_fwdcolor=cyan
                /ApEinfo_revcolor=#00ff00
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
misc_feature    9336..9336
                /label=T>C in 40A(1)
                /ApEinfo_label=T>C in 40A
                /ApEinfo_fwdcolor=cyan
                /ApEinfo_revcolor=#00ff00
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
primer_bind     5040..5057
                /label=GF9
                /ApEinfo_fwdcolor=#ff00bd
                /ApEinfo_revcolor=#ff0003
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
primer_bind     complement(7375..7398)
                /label=Sec71-GR4
                /ApEinfo_fwdcolor=#ff00bd
                /ApEinfo_revcolor=#ff0003
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
primer_bind     8137..8155
                /label=Sec71-GF13
                /ApEinfo_fwdcolor=#ff00bd
                /ApEinfo_revcolor=#ff0003
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
misc_feature    9970..9972
                /label=STOP
                /ApEinfo_fwdcolor=#66ccff
                /ApEinfo_revcolor=#00ff00
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
```

```
width 5 offset 0
primer_bind    7073..7094
               /label=Sec71-GF15
               /ApEinfo_fwdcolor=#fb53d0
               /ApEinfo_revcolor=#fc5a5d
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature   7099..7101
               /label=PAM
               /ApEinfo_fwdcolor=#fc81f0
               /ApEinfo_revcolor=#fc81f0
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature   7208..7210
               /label=PAM(1)
               /ApEinfo_label=PAM
               /ApEinfo_fwdcolor=#fc81f0
               /ApEinfo_revcolor=#fc81f0
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature   4942..4983
               /label=V5(1)
               /ApEinfo_label=V5
               /ApEinfo_fwdcolor=#ffffcc
               /ApEinfo_revcolor=#ffffcc
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature   4984..4989
               /label=SpeI
               /ApEinfo_fwdcolor=#ffffcc
               /ApEinfo_revcolor=#ffffcc
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature   4990..5013
               /label=Linker GL3
               /ApEinfo_fwdcolor=#ffcc66
```



```

    /ApEinfo_revcolor=#ffcc66
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 4892..4909
    /label=Metallothionein_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature complement(4327..4482)
    /label=lacZ_a
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
promoter 4463..4479
    /label=M13_forward20_primer
    /ApEinfo_fwdcolor=#ccffed
    /ApEinfo_revcolor=#ccffed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 4448..4470
    /label=M13_pUC_fwd_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature complement(4113..4135)
    /label=pGEX_3_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
promoter complement(3926..3954)
    /label=AmpR_promoter
    /ApEinfo_fwdcolor=#ccffed
```

```

    /ApEinfo_revcolor=#ccffed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
gene      complement(3024..3884)
    /gene="Ampicillin"
    /label=Ampicillin
    /ApEinfo_fwdcolor=#ffffcc
    /ApEinfo_revcolor=#ffffcc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
rep_origin complement(2250..2869)

/translacion="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGY
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHVTRL
DRWEPELNEAIPNDERDTMPVAMATTLRKLTTGELLTLASRQQLIDWMEADKVAGPL
LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
EIGASLIKHW*"
    /label=pBR322_origin
    /ApEinfo_fwdcolor=pink
    /ApEinfo_revcolor=pink
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
promoter  complement(1912..1941)

/translacion="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGY
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHVTRL
DRWEPELNEAIPNDERDTMPVAMATTLRKLTTGELLTLASRQQLIDWMEADKVAGPL
```

```
LRNALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
    EIGASLIKHW*"
    /label=lac_promoter
    /ApEinfo_fwdcolor=#ccffed
    /ApEinfo_revcolor=#ccffed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    complement(1876..1898)

/translation="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGY
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHSVTRL
DRWEPELNEAIPNDERDTTMPVAMATTLRKLTTGELLTLASRQQLIDWMEADKLVAGPL

LRNALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
    EIGASLIKHW*"
    /label=M13_pUC_rev_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
promoter        complement(1859..1877)

/translation="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGY
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHSVTRL
DRWEPELNEAIPNDERDTTMPVAMATTLRKLTTGELLTLASRQQLIDWMEADKLVAGPL

LRNALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
```

```
EIGASLIKHW*"
  /label=M13_reverse_primer
  /ApEinfo_fwdcolor=#ccffed
  /ApEinfo_revcolor=#ccffed
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 4489..4856
  /label=MT-promoter
  /ApEinfo_fwdcolor=cyan
  /ApEinfo_revcolor=#00ff00
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature complement(1350..1708)
  /label=Copia Promoter?
  /ApEinfo_fwdcolor=#cde7f7
  /ApEinfo_revcolor=#cde7f7
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind 4462..4479
  /label=M13-fwd
  /ApEinfo_fwdcolor=cyan
  /ApEinfo_revcolor=green
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind complement(1859..1879)
  /label=M13-rev
  /ApEinfo_fwdcolor=cyan
  /ApEinfo_revcolor=green
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
rep_origin complement(2247..2929)
  /label=ColE1 origin
  /ApEinfo_fwdcolor=gray50
  /ApEinfo_revcolor=gray50
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
```

```
CDS      complement(4323..4391)
         /label=LacZ alpha
         /ApEinfo_fwdcolor=#6495ed
         /ApEinfo_revcolor=#6495ed
         /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
         width 5 offset 0
misc_binding  complement(1885..1907)
         /label=Lac0
         /ApEinfo_fwdcolor=#6495ed
         /ApEinfo_revcolor=#6495ed
         /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
         width 5 offset 0
CDS      complement(3027..3686)
         /label=AmpR
         /ApEinfo_fwdcolor=yellow
         /ApEinfo_revcolor=yellow
         /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
         width 5 offset 0
primer_bind  4436..4459
         /label=M13F
         /ApEinfo_fwdcolor=#0a00ff
         /ApEinfo_revcolor=#0a00ff
         /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
         width 5 offset 0
primer_bind  4462..4479
         /label=M13F_GW
         /ApEinfo_fwdcolor=#ff00bd
         /ApEinfo_revcolor=#ff0003
         /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
         width 5 offset 0
primer_bind  complement(1859..1879)
         /label=M13R_GW
         /ApEinfo_fwdcolor=#ff00bd
         /ApEinfo_revcolor=#ff0003
         /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
         width 5 offset 0
```

```
primer_bind    3998..4015
               /label=pQE60-F
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
primer_bind    complement(3605..3629)
               /label=Amp-GF
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
primer_bind    3147..3170
               /label=Amp-GR1
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
misc_feature   complement(225..1250)
               /label=Hyg
               /ApEinfo_fwdcolor=#ffffcc
               /ApEinfo_revcolor=#00ff00
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
misc_feature   1254..1268
               /label=New Feature(1)
               /ApEinfo_label=New Feature
               /ApEinfo_fwdcolor=cyan
               /ApEinfo_revcolor=#00ff00
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
gene           complement(143..173)
               /gene="puro (variant)"

/translation="MGSCAPFGRALRVVGRASGTGLAGHAPGARSFGHLDVGGDGEAE
```

```
PLVEGEVAGRGLQEGGHPGALGRLHSGEHDGAAQTLALVVGRDADGGQEPRGLLGPV

RRQEAFHLLLRGQPGTAQLGHARADLGEHRPRFDALRRGPDRHRGAVVRDPHLADVEP

DAREEEFLQLGDPLDVAVRVDGVARGGVVGERGGEGAYGPGDVVAGGEAHRGLVLGHG
    RSPCEGSGAWVRGWRHRSWRPTCRHASYPARAARGICCNL*"
    /label=puro (variant)
    /ApEinfo_fwdcolor=#ffffcc
    /ApEinfo_revcolor=#ffffcc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    95..115
    /label=New Feature(2)
    /ApEinfo_label=New Feature
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0

ORIGIN
    1 ataagctgca ataaacaagt tctagagtcg gtgggcctcg ggggcgggtg cggggtcggc
    61 ggggccgcc cgggtggctt cggtcggagc catggggtcg tgcgctcctt tcggtcggc
    121 gctgcggtc gtggggcggg cgtcaggcac cgggcttgcg ggtcatgcac cagttgctgg
    181 ctgaggcgtt ctcgaaatca gctcttgttc ggtcggcatc tactctattc ctttgcctc
    241 ggacgagtcg tggggcgctg gtttccacta tcggcgagta cttctacaca gccatcggtc
    301 cagacggccg cgcttctgcg ggcgatttgt gtacgccca cagtcccggc tccggatcgg
    361 acgattgcgt cgcacgacc ctgcgcccga gctgcatcat cgaaattgcc gtcaaccaag
    421 ctctgataga gttggtcaag accaatgcgg agcatatacg cccggagccg cggcgatcct
    481 gcaagctccg gatgcctccg ctcgaagtag cgcgtctgct gctccataca agccaaccac
    541 ggctccaga agaagatggt ggcgacctc gattgggaat ccccgaacat cgcctcgtc
    601 cagtcaatga ccgctgttat gcggccattg tccgtcagga cattgttggg gccgaaatcc
    661 gcgtgcacga ggtgccggac ttcggggcag tcctcggccc aaagcatcag ctcatcgaga
    721 gcctgcgcga cggacgcact gacggtgctg tccatcacag tttgccagtg atacacatgg
    781 ggatcagcaa tcgcgcatat gaaatcacgc catgtagtgt attgaccgat tccttgcggt
    841 ccgaatgggc cgaacccgct cgtctggcta agatcggccg cagcgatcgc atccatggcc
    901 tccgcgaccg gctgcagaac agcgggcagt tcggtttcag gcaggctctg caacgtgaca
    961 ccctgtgcac ggcgggagat gcaataggtc aggctctcgc tgaattcccc aatgtcaagc
```



1021 acttccggaa tcgggagcgc ggccgatgca aagtgccgat aaacataacg atctttgtag  
1081 aaaccatcgg cgcagctatt taccgcgagg acatatccac gccctcctac atcgaagctg  
1141 aaagcacgag attcttcgcc ctccgagagc tgcatacagg cggagacgct gtcgaacttt  
1201 tcgatcagaa acttctcgac agacgtcgcg gtgagttcag gctttttcat ggtggtcgtc  
1261 tccttgtgag gggtcagggg cgtgggtcag gggatggtgg cggcaccggt cgtggcggcc  
1321 gacctgcagg catgcaagct atcgaattcc tgcagcccgg gggatctgtt gtaatttata  
1381 atttatattt cttttcttaa taaataaata aatagtcaag tttatgttg agttttatga  
1441 tttatatttt aagttatttc aactgcaaca ccagcaccac gacctactta cagcaaaaaa  
1501 cgtacaagaa ggaagaagg aataaaaaga gtggtattct cttacaatat gttttatggc  
1561 ataaaagggt tggccattca tatcaaatat aaagtagtgt tgtttaacgt tacttttcta  
1621 ggttgaatag tatattccaa cagatgatga ggggttccca atcctaacc catttgccgt  
1681 tcccagaagc atgaaaccac cacgcaccgg atcctctaga acaacaacaa ttgcattcat  
1741 tttatgtttc aggttcaggg ggaggtgtgg gaggtttttt aaagcaagta aaacctctac  
1801 aatgttggtg tggctgatta tgatcagtcg acctgcaggc atgcaagctt ggcgtaatca  
1861 tggcatagc tgtttcctgt gtgaaattgt tatccgctca caattccaca caacatacga  
1921 gccggaagca taaagtgtaa agcctggggg gcctaagtag tgagctaact cacattaatt  
1981 gcgttgcgct cactgcccgc tttccagtcg ggaacctgt cgtgccagct gcattaatga  
2041 atcggccaac gcgcggggag aggcggtttg cgtattgggc gctcttccgc ttctctgctc  
2101 actgactcgc tgcgctcggg cgttcggctg cggcgagcgg tatcagctca ctcaaaggcg  
2161 gtaatacggg tatccacaga atcaggggat aacgcaggaa agaacatgtg agcaaaaggc  
2221 cagcaaaagg ccaggaaccg taaaaggcc gcgttgctgg cgtttttcca taggctccgc  
2281 cccctgacg agcatcaca aaatcgacgc tcaagtcaga ggtggcgaaa cccgacagga  
2341 ctataaagat accaggcgtt tccccctgga agctccctcg tgcgctctcc tgttccgacc  
2401 ctgccgctta ccggatacct gtccgccttt ctcccttcgg gaagcgtggc gcttttctcat  
2461 agctcacgct gtaggtatct cagttcgggt taggtcgttc gctccaagct gggctgtgtg  
2521 cacgaacccc ccgttcagcc cgaccgctgc gccttatccg gtaactatcg tcttgagtcc  
2581 aacccggtaa gacacgactt atcgccactg gcagcagcca ctggtaacag gattagcaga  
2641 gcgaggtatg taggcggtgc tacagagttc ttgaagtggg ggcctaacta cggctacact  
2701 agaagaacag tatttggtat ctgcgctctg ctgaagccag ttaccttcgg aaaaagagtt  
2761 ggtagctctt gatccggcaa acaaacacc gctggtagcg gtggtttttt tgtttgcaag  
2821 cagcagatta cgcgcagaaa aaaaggatct caagaagatc ctttgatctt ttctacgggg  
2881 tctgacgctc agtggaacga aaactcacgt taagggattt tggatcatgag attatcaaaa  
2941 aggatcttca cctagatcct tttaaattaa aatgaagtt ttaaatcaat ctaaagtata  
3001 tatgagtaaa cttggtctga cagttaccaa tgcttaatca gtgaggcacc tatctcagcg  
3061 atctgtctat ttcggtcatc catagttgcc tgactccccg tcgtgtagat aactacgata  
3121 cgggagggct taccatctgg cccagtgct gcaatgatac cgcgagacc acgctcaccg

3181 gctccagatt tatcagcaat aaaccagcca gccggaaggg ccgagcgag aagtggctct  
3241 gcaactttat ccgcctccat ccagtctatt aattgttgcc ggaagctag agtaagtagt  
3301 tcgccagtta atagtttgcg caacgttggt gccattgcta caggcatcgt ggtgtcacgc  
3361 tcgtcgtttg gtatggcttc attcagctcc ggttcccaac gatcaaggcg agttacatga  
3421 tccccatgt tgtgcaaaaa agcggttagc tccttcggtc ctccgatcgt tgtcagaagt  
3481 aagttggccg cagtgttatc actcatggtt atggcagcac tgcataattc tcttactgtc  
3541 atgccatccg taagatgctt ttctgtgact ggtgagtact caaccaagtc attctgagaa  
3601 tagtgtatgc ggcgaccgag ttgctcttgc ccggcgtaa tacgggataa taccgcgcca  
3661 catagcagaa ctttaaaagt gctcatcatt ggaaaacgtt ctccggggcg aaaactctca  
3721 aggatcttac cgctgttgag atccagttcg atgtaacca ctctgtcacc caactgatct  
3781 tcagcatctt ttactttcac cagcgtttct gggtgagcaa aaacaggaag gcaaaatgcc  
3841 gcaaaaaagg gaataagggc gacacggaaa tgttgaatac tcatactctt ctttttcaa  
3901 tattattgaa gcatttatca gggttattgt ctcatgagcg gatacatatt tgaatgtatt  
3961 tagaaaaata aacaaatagg ggttccgcgc acatttccc gaaaagtgcc acctgacgtc  
4021 taagaaacca ttattatcat gacattaacc tataaaaata ggcgtatcac gaggcccttt  
4081 cgtctcgcgc gtttcggtga tgacgggtga aacctctgac acatgcagct cccggagacg  
4141 gtcacagctt gtctgtaagc ggatgccggg agcagacaag cccgtcaggg cgcgtcagcg  
4201 ggtgttgggc ggtgtcgggg ctggcttaac tatgcggcat cagagcagat tgtactgaga  
4261 gtgcaccata tgcggtgtga aataccgcac agatgcgtaa ggagaaaata ccgcatcagg  
4321 cgccattcgc cattcaggct gcgcaactgt tgggaagggc gatcgggtcg ggcctcttcg  
4381 ctattacgcc agctggcgaa aggggatgt gctgcaaggc gattaagttg ggtaacgcca  
4441 gggttttccc agtcacgacg ttgtaaaacg acggccagt aattaattcg ttgcaggaca  
4501 ggatgtggtg cccgatgtga ctagctcttt gctgcaggcc gtcctatcct ctggttcga  
4561 taagagaccc agaactccgg cccccaccg cccaccgcca ccccataca tatgtgttac  
4621 gcaagtaaga gtgcctcgc atgccccatg tgccccacca agagctttgc atccataca  
4681 agtcccaaa gtggagaacc gaaccaattc ttcgaggca gaacaaaagc ttctgcacac  
4741 gtctccactc gaatttgag ccggccggcg tgtgcaaaag aggtgaatcg aacgaaagac  
4801 ccgtgtgtaa agcccggtt caaaatgta taaaaccgag agcatctggc caatgtgcat  
4861 cagttgtggt cagcagcaaa atcaagtga tcatctcagt gcaactaaag ggggatcta  
4921 gatcggggta ccgccaccAT GGGTAAGCCT ATCCCTAACC CTCTCCTCGG TCTCGATTCT  
4981 ACGACTAGTG GAGGAGGAGG TTCTGGTGGT GGTACAACA ACTCCACAAA AACCAAGGAA  
5041 ATGTTTCATCG TGCGTGTCT AGAAAAGATC CTTGCCGATA AGGACATACG GCGTCCCAT  
5101 CACTCGCAGC TGAAGAAGTC CTGCGATTCT GCGCTGGAGC AGATTAAGGC GGAGCTAATC  
5161 AGTGCCGGCC AGATCGCAGA GGGCAATGAG CTGCCCTGTG CCGCACTCCC GCTGCCAAG  
5221 AATGATGCAG CGAGCATCAT AAATGCGGAG ACCTACTTTC TCCCCTTCGA GCTTGCCTGC  
5281 AAGAGCCGCT CGCCCAGGAT CGTGGTCACC GCACTGGACT GCCTGCAGAA ACTCATTGCC

5341 TATGGCCATT TGACAGGATC CATTGAGGAC TCGGCCAATC CGGGTCACCT GCTCATCGAC  
5401 CGTATCGTTG TGACCATATA TGGCTGCTTC AGTGGTCCCC AGACGGACGA GGCCGTCCAA  
5461 CTGCAGATAA TAAAGGCTCT GCTCACGGTG GTCACCTCGC AGCATGTGGA AATCCATGAA  
5521 TTCACACTGC TGCAAGCTGT GCGCACCTGC TACGACATCT ATTTGTCCAG CAAGAACCTG  
5581 GTCAATCAGA CCACAGCACG CGCTACGCTC ACCCAAATGT TGAACGTGAT ATTTGCCCGC  
5641 ATGGAGAATC AAGTGACGA GCTACCACCT CCCAATTCCA ATCCCACCAA CGGCAGCATC  
5701 CACTCGGAGG ATTGCAATGG CTCGGGAGAG GAGTCGCTGC GGGATTCCGA CGAAGTAATT  
5761 GCCTCGGAAC TGCTGGCGGA GATCATATCA GCTGCCTACA ATGAGGCGAT GAAGGATCAG  
5821 GAATCGGTCTG GTGAGCCAGA GCCAACACTT AATGGAAACG ACTACTCCTC GCACTCGGAT  
5881 CACGACAGTG TGGAGCTGCA CAGCGAAAAC GATGCGGTTG TAACGGCTAA GTTTACGCAC  
5941 ATCCTGCAGA AAGATGCTTT TCTCGTGTTT CGGGCACTGT GCAAGCTATC GATGAAGCCT  
6001 TTGCCGGATG GACATCCAGA TCCGAAATCG CACGAGCTGC GTTCCAAGGT GCTGTCATTG  
6061 CATCTGCTGC TGCTCATCCT CCAGAATGCC GGGCCCGTCT TCCGCTCCAA CGAGATGTTT  
6121 ATCATGGCCA TTAAGCAGTA CCTGTGCGTG GCCTTGTCAC ACAACGGAGT CAGTCTGGTG  
6181 CCGGAGGTCT TCGAGCTGTC GCTTTCAATC TTCGTTGCC TACTCTCGAA CTTCAAGGTG  
6241 CATCTTAAGC GGCAGATAGA GGTGTTCTTC AAGGAAATCT TCCTAAACAT TCTTGAGGCG  
6301 AACTCAAGCA GCTTCGAGCA CAAATGGATG GTAATCCAAG CGCTGACACG TATTTGTGCT  
6361 GACGCCAGT CCGTGGTGA TATCTATGTT AATTACGATT GCGACTTTTC GGCTGCAAAC  
6421 CTTTTTGAGA GACTGGTCAA CGATCTTTCG AAAATTGCC AGGGTCGTCA GGCTCTCGAA  
6481 CTGGGCGCCA ATCCGATGCA AGAGAAATCG ATGCGCATTG GCGGCCTGGA GTGTCTTGTC  
6541 TCCATTCTTA AGTGCATGGT AGAGTGGAGT AAGGACTTGT ATGTTAATCC AAACATGCCG  
6601 GTTCCACCTA TGCAAGTCCA ATCGCCGACA AGCACTGAGC AGGATCAGGC GGACACAAC  
6661 ATCCAAACGA TGCACAGTGG TTCCAGTCAT AGTTTGAAC CCAATCAGGA GCAACTACAG  
6721 GATCTTCCCG AGGCATTGGA GGAGCGCAAG ATGCGCAAGG AAGTGATGGA AACAGGCATT  
6781 GAGTTATTCA ATCGTAAGCC TCAGAAAGGA GTGCAATTCC TGCAGGAGAA GCAGTTGCTG  
6841 GGTGCCACAT GCGGGGACAT TGCGCGCTGG CTGCACGAGG ACGAACGACT GGACAAGACA  
6901 GTGATCGGAA ACTACATTGG CGAGAATGAC GACCACTCCA AGGAAGTGAT GTGCGCTTAC  
6961 ATCGATGCCT TTGACTTTCG CCAAATGGAG GTGGTGGCCG CTTGAGATT TCTTCTCGAG  
7021 GGGTTCCGCC TGCCAGGAGA AGCACAAAAA ATCGATCGGC TGATGGAGAA GTTCGCCAGT  
7081 AGATACTGCG AATGCAATCC GAAGAACCAG CTATTCCAAA GCGCAGACAC CGTCTACGTG  
7141 CTGGCATTCA GCATCATTAT GCTGACCACG GATCTTCATT CGCCGAGGT CAAGCACAAG  
7201 ATGACCAAGG AGCAGTACAT TAAAATGAAC CGCGGCATCA GCGACAGCAA GTCCGATTG  
7261 CCCGAGGAGT ACTTGTGCTC CATCTACGAC GAGATTTCTG AACACGAAAT TAAGATGAAG  
7321 AACAACTCCG GTATGCTTCA ACAGGCGAAA CCCACTGGAA AGCAGGCCTT CATAACGGAG  
7381 AAACGAGAA AGCTGTTGTG GAACATGGAG ATGGAGGTCA TCTCGCTGAC GGCCACCAAT  
7441 CTAATGCAGT CAGTTTCGCA CGTCAAGTCA CCCTTCACCT CAGCGAAACA CTTGGAGCAT

7501 GTCCGGCCCA TGTTCAAAT GGCTTGGACA CCATTTCTGG CCGCTTTCTC TGTGGGTCTC  
7561 CAGGACTGCG ACGATCCTGA GATTGCTACA CTCTGCTTGG ATGGTATACG TTGTGCTATT  
7621 CGAATCGCAT GCATCTTCCA CATGTCCCTG GAGCGAGATG CCTATGTACA AGCCCTGGCC  
7681 AGGTTTACTC TCCTGAATGC TAACTCGCCC ATCAACGAAA TGAAGGCCAA GAATATCGAT  
7741 ACCATCAAGA CGCTTATAAT GGTAGCCAC ACGGATGGCA ATTATCTGGG CAGCAGCTGG  
7801 CTGGATATAG TGAAGTGCAT TAGCCAGTTG GAGCTGGCCC AACTGATCGG CACTGGGGTG  
7861 CGGCCCCAGT TTCTTTCTGG AGCGCAGACA ACGCTCAAGG ACTCGCTTAA TCCCAGCGTG  
7921 AAAGAACACA TCGGCGAGAC GAGCAGCCAG AGCGTGGTGG TCGCAGTCGA TCGTATTTTC  
7981 ACCGGCTCAA TGC GACTGGA TGGCGATGCT ATCGTGGACT TCGTGAAGGC CCTGTGCCAG  
8041 GTGTCTGTGG ATGAGCTTCA GCAGCAGCAA CCGAGGATGT TCTCCTTGA AAAGATAGTG  
8101 GAAATTAGTT ACTACAACAT GGAGCGTATT CGTCTGCAGT GGTCACGCAT TTGGCAAGTT  
8161 TTGGGTGAGC ACTTTAACGC GGTCGGATGC AATAGCAACG AGGAGATCTC ATTTTTCGCC  
8221 CTGGACTCAC TGC GTCAGTT GTCGATGAAG TTCATGGAGA AGGGCGAGTT CAGTAATTTT  
8281 CGCTTCCAGA AGGATTTCTT GCGTCCCTTT GAGCATATCA TGAAGAAAAA CGCATCGCCG  
8341 GCAATACGAG ATATGGTGGT GCGCTGCATT GCCCAGATGG TAAACTCACA GGCGCATAAC  
8401 ATCCGTTCCG GCTGGAAGAA TATCTTTAGC ATTTTCCACC TGGCAGCGGG AGACAACGAA  
8461 GAGCCAATTG TGGAGCTGGC CTTCAAACC ACGGGCAAAA TCATCGGTGA TCTGTACAAG  
8521 CGTCAGTTCC CCATTATGGT GGACTCGTTC CAGGATGCGG TCAAGTGCCT GTCAGAGTTC  
8581 GCCACCGCCA GATTCCCCGA TACCAGCATG GAAGCCATAC GTCTGGTCCG TACCTGCGCG  
8641 CAGTGCCTCC ACGAGGCACC ACAACTGTTT GCGGAGCATG CCGGCATGGA GAACGACGCC  
8701 TCGGTGGCCG AGGAGGATCG AGTCTGGGTG CGCGGCTGGT TTCCGATGCT ATTCTCGCTT  
8761 TCCTGCGTGG TCAATCGCTG CAAATTGGAT GTGCGTACTC GCGCCTTAAC CGTGCTTTTT  
8821 GAGATTGTGA AGACGTATGG TGAGAGCTTC AAGCCCCATT GGTGGAAGGA TCTCTTCAAT  
8881 GTGATCTTCC GTATCTTCGA CAACATGAAA TTGCCGGAGC ACGTCACCGA GAAGTCCGAA  
8941 TGGATGACGA CCACATGCAA CCACGCCTTG TACGCTATTA TTGATGTCTT CACGCAGTAT  
9001 TTCGATGTTC TTGGTCATCT GCTGCTGGAG GAGCTCTTCG CCCAGCTGCA TTGGTGTGTT  
9061 CAGCAGAGTA ACGAGCAGTT GCGCGATCT GGCACCAATT GCCTGGAGAA CCTCGTCATT  
9121 TCGAATGGAT TCAAGTTCAA CGAGTCCACC TGGGACAAGA CGTGCCAGTG CATCCTGGAC  
9181 ATCTTCAACG CCACTTTGCC GCAGGATCTC CTCAGTTGGC GGCCGAAAGC ACATTCCAGT  
9241 AACAAATATAC CCCAGGAGCA CAACCACTTT GAGGCGCTGC ATATCCGCTG CGTAGTCCAG  
9301 CTGGAAGTGA TACAGACCAT GGATAACATT GTCTTTTTCC CGGCCACGTC GCGCAAGGAG  
9361 GATGCCGAAA CGCTGGCCCA GCGGCGGCA GACTTAACAG GCGGCAGGAG CGGTTCGCAG  
9421 TCGCAGCTGC TGGAGTGCCA GCGGGAGGAG CAGGGAATGT ACGGCTATCT GAGAACCCGC  
9481 CAGCTGCTCA CCCTGGCCGA CTGTCTGATG CAGTCGCACC GTTTTGCCAA GCGCTTCAAC  
9541 GCCGATCACG ACCAACGCAG CCTGCTTTGG CGGGCGGGAT TCAAGGGATC TGTTAAACCG  
9601 AATCTGCTGA AGCAGGAGAC CTCGTCGCTG GCCTGCGTCC TGCGCATTTC CTCAAGATG

<p>9661 TACGGCGACG AGAATAGACG CAGCGATTGG CCCGGCATCG AGCAGGAACT GGTGCAGGTC            9721 TGCAAGGAGG CACTGGGCTA CTATTTGAGT TTGAGAGCG AGGCACACCG AGATGCGTGG            9781 ACATCGCTGC TGCTGCTCAT CCTGACGCGC CTGCTCAAGA TGTCCGATGC CAGGTTCCGC            9841 ACCCACGTTT CCAACTACTA CAGCCTGCTG TGCGAGATGA TGTGCTTCGA CCTCAAGCCC            9901 GAACTGAGAA GTGTCCTTAG GCGTGTGTTT ATGCGCATCG GTCCAGTATT CAATATAATG            9961 AGCGTTAAAT AAttctagtc gaccatgaag atcaagatca ttgccccgcc agagcgcaag            10021 tactctgtct gggcccttcg aaGGTAAGCC TATCCCTAAC CCTCTCCTCG GTCTCGATTC            10081 TACGcgtacc ggtCATCATC ACCATCACCA TTGAgtttaa acccgctgat cagcctcgac            10141 tgtgccttct aagatccaga catgataaga tacattgatg agtttggaca aaccacaact            10201 agaatgcagt gaaaaaatg ctttatttgt gaaatttgtg atgctattgc tttatttga            10261 accatt</p> <p>//</p>					
<p><b>pMT-hyg-V5::Sec71<sup>E677K</sup></b></p>					
LOCUS	pMT_hyg_V5_Sec71_E677K	10266 bp	ds-DNA	circular	28-FEB-2020
DEFINITION	pMT-puro Sequencing Result				
ORGANISM	other sequences; artificial sequences; vectors.				
COMMENT	pMT-vAX2m from 1 to 5573				
COMMENT	pMT-vS0G4m from 1 to 5126				
COMMENT	pMT-mS0Gm_v4 from 1 to 5114				
COMMENT	ApEinfo:methylated:1				
FEATURES	Location/Qualifiers				
misc_feature	complement(10177..10196)				
	/label=EBV_rev_primer				
	/ApEinfo_fwdcolor=#ff3600				
	/ApEinfo_revcolor=#ff3600				
	/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}				
	width 5 offset 0				
misc_feature	complement(10134..10151)				
	/label=BGH_rev_primer				
	/ApEinfo_fwdcolor=#ff3600				
	/ApEinfo_revcolor=#ff3600				
	/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}				
	width 5 offset 0				
misc_feature	4892..4909				
	/label=Metallothionein_primer				

```
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature 9363..9363
    /label=T>C silent
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature 10094..10111
    /label=6xHis
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature 10043..10084
    /label=V5
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature 10031..10036
    /label=ApaI GGGCC^C
    /ApEinfo_fwdcolor=#ffffcc
    /ApEinfo_revcolor=#ffffcc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
CDS 6600..6600
    /label=Sec71-PA
    /ApEinfo_fwdcolor=#99ccff
    /ApEinfo_revcolor=#cde7f7
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature 9447..9447
    /label=G>A silent
```

```

/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 4927..4932
/label=KpnI
/ApEinfo_fwdcolor=#ffffcc
/ApEinfo_revcolor=#ffffcc
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 9552..9552
/label=C>T silent
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
promoter complement(3926..3954)
/label=AmpR_promoter
/ApEinfo_fwdcolor=#ccffed
/ApEinfo_revcolor=#ccffed
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 9751..9751
/label=T>C silent(1)
/ApEinfo_label=T>C silent
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
gene complement(3024..3884)
/gene="Ampicillin"
/label=Ampicillin
/ApEinfo_fwdcolor=#ffffcc
/ApEinfo_revcolor=#ffffcc
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
```



```
primer_bind    6550..6569
               /label=Sec71-GF5
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
rep_origin     complement(2250..2869)

/translacion="MSIQHFRVALIPFFAAFCPLVFAHPETLVKVKDAEDQLGARVGY
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHVTRL
DRWEPELNEAIPNDERDTMPVAMATTLRKLTLGELLTLASRQQLIDWMEADKVAGPL
LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
EIGASLIKHW*"
               /label=pBR322_origin
               /ApEinfo_fwdcolor=pink
               /ApEinfo_revcolor=pink
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
misc_feature   9324..9324
               /label=T>C silent(2)
               /ApEinfo_label=T>C silent
               /ApEinfo_fwdcolor=cyan
               /ApEinfo_revcolor=#00ff00
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
promoter       complement(1912..1941)

/translacion="MSIQHFRVALIPFFAAFCPLVFAHPETLVKVKDAEDQLGARVGY
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
```

```
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLTTIGGPKELTAFLHNMGDHVTRL  
  
DRWEPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPL  
  
LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA  
    EIGASLIKHW*"  
    /label=lac_promoter  
    /ApEinfo_fwdcolor=#ccffed  
    /ApEinfo_revcolor=#ccffed  
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}  
width 5 offset 0  
primer_bind    9054..9073  
    /label=sec71-GF8  
    /ApEinfo_fwdcolor=#ff00bd  
    /ApEinfo_revcolor=#ff0003  
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}  
width 5 offset 0  
misc_feature    complement(1876..1898)  
  
/translation="MSIQHFRVALIPFFAAFLPVAHPETLVKVKDAEDQLGARVGY  
  
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE  
  
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLTTIGGPKELTAFLHNMGDHVTRL  
  
DRWEPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPL  
  
LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA  
    EIGASLIKHW*"  
    /label=M13_pUC_rev_primer  
    /ApEinfo_fwdcolor=#ff3600  
    /ApEinfo_revcolor=#ff3600  
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}  
width 5 offset 0  
primer_bind    5924..5943  
    /label=sec71-GF4
```

```

    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
promoter      complement(1859..1877)

/translacion="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGY
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHVTRL
DRWEPELNEAIPNDERDTMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPL
LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
    EIGASLIKHW*"
    /label=M13_reverse_primer
    /ApEinfo_fwdcolor=#ccffed
    /ApEinfo_revcolor=#ccffed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature  7039..7041
    /label=E677 (E740 in garz)
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature  10011..10030
    /label=New Feature
    /ApEinfo_fwdcolor=#ffffcc
    /ApEinfo_revcolor=#ffffcc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature  6069..6069
    /label=C>T confirmed in 40A 2013
    /ApEinfo_fwdcolor=cyan
```

```

/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    9662..9679
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    9662..9679
/ApEinfo_fwdcolor=#ff00bd
/ApEinfo_revcolor=#ff0003
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    7197..7215
/ApEinfo_fwdcolor=#ff00bd
/ApEinfo_revcolor=#ff0003
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    7197..7215
/ApEinfo_fwdcolor=#ff00bd
/ApEinfo_revcolor=#ff0003
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    complement(7375..7398)
/ApEinfo_fwdcolor=#ff00bd
/ApEinfo_revcolor=#ff0003
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    complement(7375..7398)
/ApEinfo_fwdcolor=#ff00bd
/ApEinfo_revcolor=#ff0003
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    complement(1859..1879)
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=green
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    complement(1859..1879)
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=green
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    8137..8155
/ApEinfo_fwdcolor=#ff00bd
/ApEinfo_revcolor=#ff0003
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    8137..8155
/ApEinfo_fwdcolor=#ff00bd
/ApEinfo_revcolor=#ff0003
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
rep_origin    complement(2247..2929)
/ApEinfo_fwdcolor=gray50

```

```
    /ApEinfo_revcolor=gray50
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_binding    complement(1885..1907)
    /label=Lac0
    /ApEinfo_fwdcolor=#6495ed
    /ApEinfo_revcolor=#6495ed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
CDS            complement(3027..3686)
    /label=AmpR
    /ApEinfo_fwdcolor=yellow
    /ApEinfo_revcolor=yellow
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    complement(1859..1879)
    /label=M13R_GW
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    3998..4015
    /label=pQE60-F
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    6903..6925
    /label=Sec71-GF14
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    complement(3605..3629)
    /label=Amp-GF
    /ApEinfo_fwdcolor=#ff00bd
```

```

    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature    complement(7099..7121)
    /label=Sec71-crRNA7(Protospacer)
    /ApEinfo_fwdcolor=#ccff66
    /ApEinfo_revcolor=#ffff9f
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind    3147..3170
    /label=Amp-GR1
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature    7188..7207
    /label=Sec71-crRNA8 Protospacer
    /ApEinfo_fwdcolor=#ccff66
    /ApEinfo_revcolor=#ffffcc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature    4942..4983
    /label=V5(1)
    /ApEinfo_label=V5
    /ApEinfo_fwdcolor=#ffffcc
    /ApEinfo_revcolor=#ffffcc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
CDS             7890..7890
    /label=Sec71-PA(1)
    /ApEinfo_label=Sec71-PA
    /ApEinfo_fwdcolor=#99ccff
    /ApEinfo_revcolor=#cde7f7
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature    4984..4989
```

```

        /label=SpeI
        /ApEinfo_fwdcolor=#ffffcc
        /ApEinfo_revcolor=#ffffcc
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
misc_feature    7147..7149
        /label=F190 F190Y=BFA-hypersensitive
        /label=F713Y(BFA-sensitive)
        /ApEinfo_fwdcolor=cyan
        /ApEinfo_revcolor=#00ff00
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
misc_feature    4990..5013
        /label=Linker GL3
        /ApEinfo_fwdcolor=#ffcc66
        /ApEinfo_revcolor=#ffcc66
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
CDS             5014..9969
        /label=Sec71
        /ApEinfo_fwdcolor=#99ccff
        /ApEinfo_revcolor=#cde7f7
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
CDS             5977..5979
        /label=Sec71-PA(2)
        /ApEinfo_label=Sec71-PA
        /ApEinfo_fwdcolor=#99ccff
        /ApEinfo_revcolor=#cde7f7
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
misc_feature    9567..9567
        /label=T>G silent
        /ApEinfo_fwdcolor=cyan
        /ApEinfo_revcolor=#00ff00
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
```



```
width 5 offset 0
misc_feature 8509..8530
  /label=Chang-F1
  /ApEinfo_fwdcolor=cyan
  /ApEinfo_revcolor=#00ff00
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind 7770..7789
  /label=sec71-GF6
  /ApEinfo_fwdcolor=#ff00bd
  /ApEinfo_revcolor=#ff0003
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind 5568..5587
  /label=sec71-GF3
  /ApEinfo_fwdcolor=#ff00bd
  /ApEinfo_revcolor=#ff0003
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 7890..7890
  /label=A>C silent
  /ApEinfo_fwdcolor=cyan
  /ApEinfo_revcolor=#00ff00
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 7629..7629
  /label=A>G in 40A
  /ApEinfo_fwdcolor=cyan
  /ApEinfo_revcolor=#00ff00
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
CDS 6069..6069
  /label=Sec71-PA(3)
  /ApEinfo_label=Sec71-PA
  /ApEinfo_fwdcolor=#99ccff
  /ApEinfo_revcolor=#cde7f7
```

```
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
protein_bind    6733..7305
    /label=Sec7 domain
    /ApEinfo_fwdcolor=#0080ff
    /ApEinfo_revcolor=#0080ff
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    7150..7152
    /label=S191
    /ApEinfo_fwdcolor=#66ff66
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind     7250..7273
    /label=Sec71-DRSC01893-F (8A10)
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind     complement(7726..7761)
    /label=Sec71-DRSC01893-R
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind     8509..8530
    /label=sec71-GF7
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind     complement(9224..9245)
    /label=Chang-R1
    /ApEinfo_fwdcolor=#fb53d0
    /ApEinfo_revcolor=#fc5a5d
```

```
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    complement(8562..8583)
    /label=Sec71-GR3
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    6600..6600
    /label=G>A silent(1)
    /ApEinfo_label=G>A silent
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    7557..7557
    /label=T>C in 40A
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    7668..7668
    /label=A>C in 40A
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    7719..7719
    /label=A>G 40A
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    9336..9336
    /label=T>C in 40A(1)
    /ApEinfo_label=T>C in 40A
```

```
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    5040..5057
    /label=GF9
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    6797..6820
    /label=Sec71-GF12
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature   7159..7161
    /label=M717(M194 in sec7 domain M>L = BFA-resistant)
    /label=M717L(BFA-resistant)
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature   9970..9972
    /label=STOP
    /ApEinfo_fwdcolor=#66ccff
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    7073..7094
    /label=Sec71-GF15
    /ApEinfo_fwdcolor=#fb53d0
    /ApEinfo_revcolor=#fc5a5d
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature   7099..7101
```

```

        /label=PAM
        /ApEinfo_fwdcolor=#fc81f0
        /ApEinfo_revcolor=#fc81f0
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
misc_feature    7208..7210
        /label=PAM(1)
        /ApEinfo_label=PAM
        /ApEinfo_fwdcolor=#fc81f0
        /ApEinfo_revcolor=#fc81f0
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
misc_feature    7828..7828
        /label=T>C Val>Ala in FRT40A
        /ApEinfo_fwdcolor=#ccff66
        /ApEinfo_revcolor=#00ff00
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
misc_feature    complement(1350..1708)
        /label=Copia Promoter?
        /ApEinfo_fwdcolor=#cde7f7
        /ApEinfo_revcolor=#cde7f7
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
misc_feature    complement(225..1250)
        /label=Hyg
        /ApEinfo_fwdcolor=#ffffcc
        /ApEinfo_revcolor=#00ff00
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
misc_feature    1254..1268
        /label=New Feature(1)
        /ApEinfo_label=New Feature
        /ApEinfo_fwdcolor=cyan
        /ApEinfo_revcolor=#00ff00
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
```

```
width 5 offset 0
gene      complement(143..173)
          /gene="puro (variant)"

/translacion="MGSCAPFGRALRVVGRASGTGLAGHAPGARSFGHLDVGGDGEAE
PLVEGEVAGRGGLEGGHPGALGRLHSGEHDGAAQTLALVVRDADGGQEPRGLLGPV
RRQEAFHLLLRGQPSTAQLGHARADLGEHRPRFDALRRGPDRHRGAVVRDPHLADVEP
DAREEEFLQLGDPLDVAVRVDGVARGGVVGERGGEGAYGPGDVGGEAHRGLVLGHG
          RSPCEGSGAWVRGWRHRSWRPTCRHASYRIPAARGICCNL*"
          /label=puro (variant)
          /ApEinfo_fwdcolor=#ffffcc
          /ApEinfo_revcolor=#ffffcc
          /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature 95..115
          /label=New Feature(2)
          /ApEinfo_label=New Feature
          /ApEinfo_fwdcolor=cyan
          /ApEinfo_revcolor=#00ff00
          /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature complement(4327..4482)
          /label=lacZ_a
          /ApEinfo_fwdcolor=#ff3600
          /ApEinfo_revcolor=#ff3600
          /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
promoter   4463..4479
          /label=M13_forward20_primer
          /ApEinfo_fwdcolor=#ccffed
          /ApEinfo_revcolor=#ccffed
          /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
```

```
misc_feature    4448..4470
                /label=M13_pUC_fwd_primer
                /ApEinfo_fwdcolor=#ff3600
                /ApEinfo_revcolor=#ff3600
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
misc_feature    complement(4113..4135)
                /label=pGEX_3_primer
                /ApEinfo_fwdcolor=#ff3600
                /ApEinfo_revcolor=#ff3600
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
misc_feature    4489..4856
                /label=MT-promoter
                /ApEinfo_fwdcolor=cyan
                /ApEinfo_revcolor=#00ff00
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
primer_bind     4462..4479
                /label=M13-fwd
                /ApEinfo_fwdcolor=cyan
                /ApEinfo_revcolor=green
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
CDS             complement(4323..4391)
                /label=LacZ alpha
                /ApEinfo_fwdcolor=#6495ed
                /ApEinfo_revcolor=#6495ed
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
primer_bind     4436..4459
                /label=M13F
                /ApEinfo_fwdcolor=#0a00ff
                /ApEinfo_revcolor=#0a00ff
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
```



```
primer_bind    4462..4479
               /label=M13F_GW
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
```

ORIGIN

```
1  ataagctgca ataaacaagt tctagagtcg gtgggcctcg ggggcgggtg cggggtcggc
61  ggggccgccc cgggtggctt cggtcggagc catggggtcg tgcgctcctt tcggtcgggc
121 gctgcgggtc gtggggcggg cgtcaggcac cgggcttgcg ggtcatgcac cagttgctgg
181 ctgaggcggt ctcgaaatca gctcttgttc ggtcggcatc tactctattc ctttgcctc
241 ggacgagtg cggggcgctg gtttccacta tcggcgagta cttctacaca gccatcggtc
301 cagacggccg cgcttctgcy ggcgatttgt gtacgccca cagtcccggc tccggatcgg
361 acgattgcyt cgcctcgacc ctgcgccca gctgcatcat cgaaattgcc gtcaaccaag
421 ctctgataga gttggtcaag accaatgcyg agcatatacy cccggagccg cggcgatcct
481 gcaagctccg gatgcctccg ctggaagtag cgcgtctgct gctccataca agccaaccac
541 ggcctccaga agaagatggt ggcgacctc gattgggaat ccccgaacat cgcctcgcct
601 cagtcaatga ccgctgttat gcggccattg tccgtcagga cattgttggg gccgaaatcc
661 gcgtgcacga ggtgccggac ttcggggcag tcctcggccc aaagcatcag ctcatcgaga
721 gcctgcgcga cggacgcact gacggtgctg tccatcacag tttgccagtg atacacatgg
781 ggatcagcaa tcgcgcatat gaaatcacgc catgtagtgt attgaccgat tccttgcggt
841 ccgaatgggc cgaacccgct cgtctggcta agatcggccg cagcgatcgc atccatggcc
901 tccgcgaccg gctgcagaac agcgggcagt tcggtttcag gcaggctctg caacgtgaca
961 ccctgtgcac ggcgggagat gcaataggtc aggctctcgc tgaattcccc aatgtcaagc
1021 acttccggaa tcgggagcgc ggcgatgca aagtgccgat aaacataacg atctttgtag
1081 aaaccatcgg cgcagctatt taccgcagg acatatccac gccctcctac atcgaagctg
1141 aaagcacgag attcttcgcc ctccgagagc tgcacaggtt cggagacgct gtcgaacttt
1201 tcgatcagaa acttctcgac agacgtcgcg gtgagttcag gctttttcat ggtggtcgtc
1261 tccttgtgag gggtcagggg cgtgggtcag gggatggtgg cggcaccggt cgtggcggcc
1321 gacctgcagg catgcaagct atcgaattcc tgcagcccgg gggatctggt gtaatttata
1381 atttatattt ctttcttaa taaataaata aatagtcag tttatgttg agttttatga
1441 tttatatttt aagttatttc aactgcaaca ccagcaccac gacctacta cagcaaaaaa
1501 cgtacaagaa ggaagaagg aataaaaaga gtggtattct cttacaatat gttttatggc
1561 ataaaagggt tggcattca tatcaaatat aaagtagtgt tgtttaacgt tacttttcta
1621 ggttgaatag tatattcaa cagatgatga ggggttccca atcctaacc catttgccgt
1681 tcccagaagc atgaaaccac cagcaccggt atcctctaga acaacaaca ttgcattcat
```

1741 tttatgtttc aggttcaggg ggaggtgtgg gaggtttttt aaagcaagta aaacctctac  
1801 aaatgtggta tggctgatta tgatcagtcg acctgcaggc atgcAAGCTT ggcgtaatca  
1861 tggcatagc tgtttcctgt gtgaaattgt tatccgctca caattccaca caacatacga  
1921 gccggaagca taaagtgtaa agcctggggg gcctaatagag tgagctaact cacattaatt  
1981 gcgttgcgct cactgcccgc tttccagtcg ggaaacctgt cgtgccagct gcattaatga  
2041 atcggccaac gcgcggggag aggcggtttg cgtattgggc gctcttccgc ttcctcgctc  
2101 actgactcgc tgcgctcggg cgttcggctg cggcgagcgg tatcagctca ctcaaaggcg  
2161 gtaatacggg tatccacaga atcaggggat aacgcaggaa agaacatgtg agcaaaaggc  
2221 cagcaaaagg ccaggaaccg taaaaaggcc gcgttgctgg cgtttttcca taggctccgc  
2281 cccctgacg agcatcacia aaatcgacgc tcaagtcaga ggtggcgaag cccgacagga  
2341 ctataaagat accaggcgtt tccccctgga agctccctcg tgcgctctcc tgttccgacc  
2401 ctgccgctta ccggatacct gtccgccttt ctcccttcgg gaagcgtggc gctttctcat  
2461 agctcacgct gtaggtatct cagttcgggtg taggtcgttc gctccaagct gggctgtgtg  
2521 cacgaacccc ccgttcagcc cgaccgctgc gccttatccg gtaactatcg tcttgagtc  
2581 aacccggtaa gacacgactt atcgccactg gcagcagcca ctggtaacag gattagcaga  
2641 gcgaggtatg taggcggtgc tacagagttc ttgaagtggg ggcctaacta cggctacact  
2701 agaagaacag tatttggtat ctgcgctctg ctgaagccag ttaccttcgg aaaaagagtt  
2761 ggtagctctt gatccggcaa acaaacacc gctggtagcg gtggtttttt tgtttgcaag  
2821 cagcagatta cgcgcagaaa aaaaggatct caagaagatc ctttgatctt ttctacgggg  
2881 tctgacgctc agtggaacga aaactcacgt taagggattt tggctatgag attatcaaaa  
2941 aggatcttca cctagatcct tttaaattaa aaatgaagtt ttaaatcaat ctaaagtata  
3001 tatgagtaaa cttggtctga cagttaccaa tgcttaatca gtgaggcacc tatctcagcg  
3061 atctgtctat ttcgttcatc catagttgcc tgactccccg tcgtgtagat aactacgata  
3121 cgggagggct taccatctgg cccagtgct gcaatgatac cgcgagacc acgctaccg  
3181 gctccagatt tatcagcaat aaaccagcca gccggaaggg ccgagcgag aagtggctct  
3241 gcaactttat ccgcctccat ccagtctatt aattgttgcc ggaagctag agtaagtagt  
3301 tcgccagtta atagtttgcg caacgttggt gccattgcta caggcatcgt ggtgtcacgc  
3361 tcgtcgtttg gtatggcttc attcagctcc gggtcccaac gatcaaggcg agttacatga  
3421 tccccatgt tgtgcaaaaa agcggtagc tccttcggtc ctccgatcgt tgtcagaagt  
3481 aagttggccg cagtgttatc actcatggtt atggcagcac tgcataattc tcttactgtc  
3541 atgccatccg taagatgctt ttctgtgact ggtgagtact caaccaagtc attctgagaa  
3601 tagtgatgac ggcgaccgag ttgctcttgc ccggcgtaac tacgggataa taccgcgcca  
3661 catagcagaa ctttaaaagt gctcatcatt ggaaaacgtt cttcggggcg aaaactctca  
3721 aggatcttac cgctggtgag atccagttcg atgtaacca ctcgtgcacc caactgatct  
3781 tcagatctt ttactttcac cagcgtttct gggtagcaa aaacaggaag gcaaaatgcc  
3841 gcaaaaaagg gaataagggc gacacggaaa tgttgaatac tcatactctt ctttttcaa

3901 tattattgaa gcatttatca gggttattgt ctcatgagcg gatacatatt tgaatgtatt  
3961 tagaaaaata aacaaatagg ggttccgcgc acatttcccc gaaaagtgcc acctgacgtc  
4021 taagaaacca ttattatcat gacattaacc tataaaaaata ggcgtatcac gaggcccttt  
4081 cgtctcgcgc gtttcggtga tgacgggtgaa aacctctgac acatgcagct cccggagacg  
4141 gtcacagctt gtctgtaagc ggatgccggg agcagacaag cccgtcaggg cgcgtcagcg  
4201 ggtgttggcg ggtgtcgggg ctggcttaac tatgcgcat cagagcagat tgtactgaga  
4261 gtgcaccata tgcggtgtga aataccgcac agatgcgtaa ggagaaaata ccgcatcagg  
4321 cgccattcgc cattcaggct gcgcaactgt tgggaagggc gatcgggtgcg ggcctcttcg  
4381 ctattacgcc agctggcgaa agggggatgt gctgcaaggc gattaagttg ggtaacgcca  
4441 gggttttccc agtcacgacg ttgtaaaacg acggccagtg aattaattcg ttgcaggaca  
4501 ggatgtggtg cccgatgtga ctagctcttt gctgcaggcc gtcctatcct ctggttccga  
4561 taagagaccc agaactccgg cccccaccg cccaccgcca ccccatatac tatgtggtac  
4621 gcaagtaaga gtgcctgcgc atgccccatg tgccccacca agagctttgc atcccatatac  
4681 agtccccaaa gtggagaacc gaaccaattc ttcgcgggca gaacaaaagc ttctgcacac  
4741 gtctccactc gaatttggag ccggccggcg tgtgcaaaag aggtgaatcg aacgaaagac  
4801 ccgtgtgtaa agccgcgttt caaaaatgta taaaaccgag agcatctggc caatgtgcat  
4861 cagttgtggt cagcagcaaa atcaagtga tcatctcagt gcaactaaag ggggatcta  
4921 gatcggggta ccgccaccAT GGGTAAGCCT ATCCCTAACC CTCTCCTCGG TCTCGATTCT  
4981 ACGACTAGTG GAGGAGGAGG TTCTGGTGGT GGTCACAACA ACTCCACAAA AACCAAGGAA  
5041 ATGTTTCATCG TCGTGCTCT AGAAAAGATC CTTGCCGATA AGGACATACG GCGCTCCCAT  
5101 CACTCGCAGC TGAAGAAGTC CTGCGATTCT GCGCTGGAGC AGATTAAGGC GGAGCTAATC  
5161 AGTGCCGGCC AGATCGCAGA GGGCAATGAG CTGCCCTGTG CCGCACTCCC GCTGCCAAG  
5221 AATGATGCAG CGAGCATCAT AAATGCGGAG ACCTACTTTC TCCCCTTCGA GCTTGCCTGC  
5281 AAGAGCCGCT CGCCCAGGAT CGTGGTCACC GCACTGGACT GCCTGCAGAA ACTCATTGCC  
5341 TATGGCCATT TGACAGGATC CATTGAGGAC TCGGCCAATC CGGGTCACCT GTCATCGAC  
5401 CGTATCGTTG TGACCATATA TGGCTGCTTC AGTGGTCCCC AGACGGACGA GGCCGTCCAA  
5461 CTGCAGATAA TAAAGGCTCT GCTCACGGTG GTCACCTCGC AGCATGTGGA AATCCATGAA  
5521 TTCACACTGC TGCAAGCTGT GCGCACCTGC TACGACATCT ATTTGTCCAG CAAGAACCTG  
5581 GTCAATCAGA CCACAGCACG CGTACGCTC ACCCAAATGT TGAACGTGAT ATTTGCCCGC  
5641 ATGGAGAATC AAGTGTACGA GCTACCACCT CCCAATTCCA ATCCCACCAA CGGCAGCATC  
5701 CACTCGGAGG ATTGCAATGG CTCGGGAGAG GAGTCGCTGC GGGATTCCGA CGAAGTAATT  
5761 GCCTCGGAAC TGCTGGCGGA GATCATATCA GCTGCCTACA ATGAGGCGAT GAAGGATCAG  
5821 GAATCGGTCTG GTGAGCCAGA GCCAACACTT AATGGAAACG ACTACTCCTC GCACTCGGAT  
5881 CACGACAGTG TGGAGCTGCA CAGCGAAAAC GATGCGGTTG TAACGGCTAA GTTTACGCAC  
5941 ATCCTGCAGA AAGATGCTTT TCTCGTGTTT CGGGCACTGT GCAAGCTATC GATGAAGCCT  
6001 TTGCCGGATG GACATCCAGA TCCGAAATCG CACGAGCTGC GTTCCAAGGT GCTGTCATTG

6061 CATCTGCTGC TGCTCATCCT CCAGAATGCC GGGCCCGTCT TCCGCTCCAA CGAGATGTTC  
6121 ATCATGGCCA TTAAGCAGTA CCTGTGCGTG GCCTTGTCAA ACAACGGAGT CAGTCTGGTG  
6181 CCGGAGGTCT TCGAGCTGTC GCTTTCAATC TTCGTTGCC TACTCTCGAA CTTCAAGGTG  
6241 CATCTTAAGC GGCAGATAGA GGTGTTCTTC AAGGAAATCT TCCTAAACAT TCTTGAGGCG  
6301 AACTCAAGCA GCTTCGAGCA CAAATGGATG GTAATCCAAG CGCTGACACG TATTTGTGCT  
6361 GACGCCCAGT CCGTGGTGA TATCTATGTT AATTACGATT GCGACTTTTC GGCTGCAAAC  
6421 CTTTTTGAGA GACTGGTCAA CGATCTTTCG AAAATTGCC AGGGTCGTCA GGCTCTCGAA  
6481 CTGGGCGCCA ATCCGATGCA AGAGAAATCG ATGCGCATT CCGGCCTGGA GTGTCTTGT  
6541 TCCATTCTTA AGTGCATGGT AGAGTGGAGT AAGGACTTGT ATGTTAATCC AAACATGCCG  
6601 GTTCCACCTA TGCAAGTCCA ATCGCCGACA AGCACTGAGC AGGATCAGGC GGACACAAC  
6661 ATCCAAACGA TGCACAGTGG TTCCAGTCAT AGTTTGAAC CCAATCAGGA GCAACTACAG  
6721 GATCTTCCC GAGCATTGGA GGAGCGCAAG ATGCGCAAGG AAGTGATGGA AACAGGCATT  
6781 GAGTTATTCA ATCGTAAGCC TCAGAAAGGA GTGCAATTCC TGCAGGAGAA GCAGTTGCTG  
6841 GGTGCCACAT GCGGGGACAT TGCGCGCTGG CTGCACGAGG ACGAACGACT GGACAAGACA  
6901 GTGATCGGAA ACTACATTGG CGAGAATGAC GACCACTCCA AGGAAGTGAT GTGCGCTTAC  
6961 ATCGATGCCT TTGACTTTCG CCAATGGAG GTGGTGGCCG CCTTGAGATT TCTTCTCGAG  
7021 GGGTCCGCC TGCCAGGAaA AGCACAAAA ATCGATCGGC TGATGGAGAA GTTCGCCAGT  
7081 AGATACTGCG AATGCAATCC GAAGAACCAG CTATTCCAAA GCGCAGACAC CGTCTACGTG  
7141 CTGGCATTCA GCATCATTAT GCTGACCACG GATCTTCATT CGCCGAGGT CAAGCACAAAG  
7201 ATGACCAAGG AGCAGTACAT TAAAATGAAC CGCGGCATCA GCGACAGCAA GTCCGATTG  
7261 CCCGAGGAGT ACTTGTCGTC CATCTACGAC GAGATTTCTG AACACGAAAT TAAGATGAAG  
7321 AACAACTCCG GTATGCTTCA ACAGGCGAAA CCCACTGGAA AGCAGGCCTT CATAACGGAG  
7381 AAACGCAGAA AGCTGTTGTG GAACATGGAG ATGGAGGTCA TCTCGCTGAC GGCCACCAAT  
7441 CTAATGCAGT CAGTTTCGCA CGTCAAGTCA CCCTTCACCT CAGCGAAACA CTTGGAGCAT  
7501 GTCCGGCCCA TGTTCAAAT GGCTTGGACA CCATTTCTGG CCGCTTCTC TGTGGGTCTC  
7561 CAGGACTGCG ACGATCCTGA GATTGCTACA CTCTGCTTGG ATGGTATACG TTGTGCTATT  
7621 CGAATCGCAT GCATCTTCCA CATGTCCCTG GAGCGAGATG CCTATGTACA AGCCCTGGCC  
7681 AGGTTTACTC TCCTGAATGC TAACTCGCCC ATCAACGAAA TGAAGGCCAA GAATATCGAT  
7741 ACCATCAAGA CGCTTATAAT GGTAGCCAC ACGGATGGCA ATTATCTGGG CAGCAGCTGG  
7801 CTGGATATAG TGAAGTGCAT TAGCCAGTTG GAGCTGGCCC AACTGATCGG CACTGGGGTG  
7861 CGGCCCCAGT TTCTTTCTGG AGCGCAGACA ACGCTCAAGG ACTCGCTTAA TCCCAGCGTG  
7921 AAAGAACACA TCGGCGAGAC GAGCAGCCAG AGCGTGGTGG TCGCAGTCGA TCGTATTTTC  
7981 ACCGGCTCAA TGCGACTGGA TGGCGATGCT ATCGTGGACT TCGTGAAGGC CCTGTGCCAG  
8041 GTGTCTGTGG ATGAGCTTCA GCAGCAGCAA CCGAGGATGT TCTCCTTGCA AAAGATAGTG  
8101 GAAATTAGTT ACTACAACAT GGAGCGTATT CGTCTGCAGT GGTCACGCAT TTGGCAAGTT  
8161 TTGGGTGAGC ACTTTAACGC GGTCGGATGC AATAGCAACG AGGAGATCTC ATTTTTCGCC

```
8221 CTGGA CTAC TGC GTCAGTT GTCGATGAAG TTCATGGAGA AGGGCGAGTT CAGTAATTTCC
8281 CGCTTCCAGA AGGATTTCTT GCGTCCCTTT GAGCATATCA TGAAGAAAAA CGCATCGCCG
8341 GCAATACGAG ATATGGTGGT GCGCTGCATT GCCCAGATGG TAAACTCACA GGCGCATAAC
8401 ATCCGTTCCG GCTGGAAGAA TATCTTTAGC ATTTTCCACC TGGCAGCGGG AGACAACGAA
8461 GAGCCAATTG TGGAGCTGGC CTTCCAAACC ACGGGCAAAA TCATCGGTGA TCTGTACAAG
8521 CGTCAGTTCC CCATTATGGT GGACTCGTTC CAGGATGCGG TCAAGTGCCT GTCAGAGTTC
8581 GCCACCGCCA GATTCCCCGA TACCAGCATG GAAGCCATAC GTCTGGTCCG TACCTGCGCG
8641 CAGTGCCTCC ACGAGGCACC ACAACTGTTT GCGGAGCATG CCGGCATGGA GAACGACGCC
8701 TCGGTGGCCG AGGAGGATCG AGTCTGGGTG CGCGGCTGGT TTCCGATGCT ATTCTCGCTT
8761 TCCTGCGTGG TCAATCGCTG CAAATTGGAT GTGCGTACTC GCGCCTTAAC CGTGCTTTTT
8821 GAGATTGTGA AGACGTATGG TGAGAGCTTC AAGCCCCATT GGTGGAAGGA TCTCTTCAAT
8881 GTGATCTTCC GTATCTTCGA CAACATGAAA TTGCCGGAGC ACGTCACCGA GAAGTCCGAA
8941 TGGATGACGA CCACATGCAA CCACGCCTTG TACGCTATTA TTGATGTCTT CACGCAGTAT
9001 TTCGATGTTT TTGGTCATCT GCTGCTGGAG GAGCTCTTCG CCCAGCTGCA TTGGTGTGTT
9061 CAGCAGAGTA ACGAGCAGTT GGCGCGATCT GGCACCAATT GCCTGGAGAA CCTCGTCATT
9121 TCGAATGGAT TCAAGTTCAA CGAGTCCACC TGGGACAAGA CGTGCCAGTG CATCCTGGAC
9181 ATCTTCAACG CCACTTTGCC GCAGGATCTC CTCAGTTGGC GGCCGAAAGC ACATTCCAGT
9241 AACAAATATAC CCCAGGAGCA CAACCACTTT GAGGCGCTGC ATATCCGCTG CGTAGTCCAG
9301 CTGGA ACTGA TACAGACCAT GGATAACATT GTCTTTTTCC CGGCCACGTC GCGCAAGGAG
9361 GATGCCGAAA CGCTGGCCCA GGCGGCGGCA GACTTAACAG GCGGCAGGAG CGGTTCCGAG
9421 TCGCAGCTGC TGGAGTGCCA GCGGGAGGAG CAGGGAATGT ACGGCTATCT GAGAACCCGC
9481 CAGCTGCTCA CCCTGGCCGA CTGTCTGATG CAGTCGCACC GTTTTGCCAA GCGCTTCAAC
9541 GCCGATCACG ACCAACGCAG CCTGCTTTGG CGGGCGGGAT TCAAGGGATC TGTTAAACCG
9601 AATCTGCTGA AGCAGGAGAC CTCGTCGCTG GCCTGCGTCC TGCGCATTTC CTTCAAGATG
9661 TACGGCGACG AGAATAGACG CAGCGATTGG CCCGGCATCG AGCAGGAACT GGTGCAGGTC
9721 TGCAAGGAGG CACTGGGCTA CTATTTGAGT TTGAGAGCG AGGCACACCG AGATGCGTGG
9781 ACATCGCTGC TGCTGCTCAT CCTGACGCGC CTGCTCAAGA TGTCCGATGC CAGGTTCCGC
9841 ACCCACGTTT CCAACTACTA CAGCCTGCTG TGCGAGATGA TGTGCTTCGA CCTCAAGCCC
9901 GAACTGAGAA GTGTCCTTAG GCGTGTGTTT ATGCGCATCG GTCCAGTATT CAATATAATG
9961 AGCGTTAAAT AAttctagtc gaccatgaag atcaagatca ttgccccgcc agagcgcaag
10021 tactctgtct gggcccttcg aaGGTAAGCC TATCCCTAAC CCTCTCCTCG GTCTCGATTC
10081 TACGcgtacc ggtCATCATC ACCATCACCA TTGAgtttaa acccgctgat cagcctcgac
10141 tgtgccttct aagatccaga catgataaga tacattgatg agtttggaca aaccacaact
10201 agaatgcagt gaaaaaatg ctttatttgt gaaatttgtg atgctattgc tttatttga
10261 accatt
```

//

pMT-hyg-V5::garz					
LOCUS	pMT_V5_garz	11146 bp	ds-DNA	circular	28-FEB-2020
DEFINITION	pMT-puro Sequencing Result				
ORGANISM	other sequences; artificial sequences; vectors.				
COMMENT	pMT-puro from 1 to 4724				
COMMENT	ApEinfo:methylated:1				
FEATURES	Location/Qualifiers				
misc_feature	complement(11057..11076)				
	/label=EBV_rev_primer				
	/ApEinfo_fwdcolor=#ff3600				
	/ApEinfo_revcolor=#ff3600				
	/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}				
	width 5 offset 0				
CDS	6913..7278				
	/label=New Feature				
	/ApEinfo_fwdcolor=#99ccff				
	/ApEinfo_revcolor=#99ccff				
	/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}				
	width 5 offset 0				
misc_feature	4942..4983				
	/label=V5				
	/ApEinfo_fwdcolor=#ffffcc				
	/ApEinfo_revcolor=#ffffcc				
	/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}				
	width 5 offset 0				
misc_feature	complement(11014..11031)				
	/label=BGH_rev_primer				
	/ApEinfo_fwdcolor=#ff3600				
	/ApEinfo_revcolor=#ff3600				
	/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}				
	width 5 offset 0				
misc_feature	complement(4327..4482)				
	/label=lacZ_a				
	/ApEinfo_fwdcolor=#ff3600				
	/ApEinfo_revcolor=#ff3600				

		/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
		width 5 offset 0
CDS	9094..10959	
		/label=garz-PB
		/ApEinfo_fwdcolor=#99ccff
		/ApEinfo_revcolor=#cde7f7
		/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
		width 5 offset 0
misc_feature	10963..10964	
		/label=V5(1)
		/ApEinfo_label=V5
		/ApEinfo_fwdcolor=cyan
		/ApEinfo_revcolor=#00ff00
		/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
		width 5 offset 0
misc_feature	7330..7332	
		/label=190Y
		/ApEinfo_fwdcolor=#ccff66
		/ApEinfo_revcolor=#00ff00
		/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
		width 5 offset 0
promoter	4463..4479	
		/label=M13_forward20_primer
		/ApEinfo_fwdcolor=#ccffed
		/ApEinfo_revcolor=#ccffed
		/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
		width 5 offset 0
CDS	5560..5586	
		/label=New Feature(1)
		/ApEinfo_label=New Feature
		/ApEinfo_fwdcolor=#99ccff
		/ApEinfo_revcolor=#99ccff
		/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
		width 5 offset 0
misc_feature	4448..4470	
		/label=M13_pUC_fwd_primer



```
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    5560..5586
    /label=JF01603-F
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    complement(4113..4135)
    /label=pGEX_3_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    8946..8982
    /label=Phosphatidylinositol-phosphate binding
    /ApEinfo_fwdcolor=#ff81f0
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
promoter        complement(3926..3954)
    /label=AmpR_promoter
    /ApEinfo_fwdcolor=#ccffed
    /ApEinfo_revcolor=#ccffed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
gene            complement(3024..3884)
    /gene="Ampicillin"
    /label=Ampicillin
    /ApEinfo_fwdcolor=#ffffcc
    /ApEinfo_revcolor=#ffffcc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
CDS            8983..9075
```

```

        /label=garz-PB(1)
        /ApEinfo_label=garz-PB
        /ApEinfo_fwdcolor=#99ccff
        /ApEinfo_revcolor=#cde7f7
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
rep_origin      complement(2250..2869)

/translacion="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGY
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHVTRL
DRWEPELNEAIPNDERDTMPVAMATTLRKLTLGELLTLASRQQLIDWMEADKVAGPL
LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
        EIGASLIKHW*"
        /label=pBR322_origin
        /ApEinfo_fwdcolor=pink
        /ApEinfo_revcolor=pink
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
CDS              9076..9093
        /label=New Feature(2)
        /ApEinfo_label=New Feature
        /ApEinfo_fwdcolor=#99ccff
        /ApEinfo_revcolor=#99ccff
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
promoter        complement(1912..1941)

/translacion="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGY
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
```

```
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLTTIGGPKELTAFLHNMGDHSVTRL  
  
DRWEPELNEAIPNDERDTMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPL  
  
LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA  
    EIGASLIKHW*"  
    /label=lac_promoter  
    /ApEinfo_fwdcolor=#ccffed  
    /ApEinfo_revcolor=#ccffed  
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}  
    width 5 offset 0  
misc_feature    4990..5013  
    /label=Linker GL3  
    /ApEinfo_fwdcolor=#ffcc66  
    /ApEinfo_revcolor=#ffcc66  
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}  
    width 5 offset 0  
misc_feature    4984..4989  
    /label=SpeI  
    /ApEinfo_fwdcolor=#ccff66  
    /ApEinfo_revcolor=#ffffcc  
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}  
    width 5 offset 0  
misc_feature    8369..8370  
    /label=MB05159  
    /ApEinfo_fwdcolor=cyan  
    /ApEinfo_revcolor=#00ff00  
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}  
    width 5 offset 0  
misc_feature    complement(1876..1898)  
  
/translation="MSIQHFRVALIPFFAAFLPVAHPETLVKVKDAEDQLGARVGY  
  
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE  
  
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLTTIGGPKELTAFLHNMGDHSVTRL
```

```
DRWEPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPL

LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
    EIGASLIKHW*"
    /label=M13_pUC_rev_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
promoter    complement(1859..1877)

/translation="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGY

IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE

YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLTTIGGPKELTAFLHNMGDHSVTRL

DRWEPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPL

LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
    EIGASLIKHW*"
    /label=M13_reverse_primer
    /ApEinfo_fwdcolor=#ccffed
    /ApEinfo_revcolor=#ccffed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
CDS        6913..7278
    /label=Sec7 domain
    EQLAKVKQKKRLLSQGTERFNQRPEKGIQYLQEHGILNAELD
    /ApEinfo_fwdcolor=#0080ff
    /ApEinfo_revcolor=#99ccff
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    4489..4856
    /label=MT-promoter
```

```

    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
CDS      6889..6912
    /label=garz-PB(2)
    /ApEinfo_label=garz-PB
    /ApEinfo_fwdcolor=#99ccff
    /ApEinfo_revcolor=#cde7f7
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind  4462..4479
    /label=M13-fwd
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=green
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind  10333..10351
    /label=garz-GF11
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind  complement(1859..1879)
    /label=M13-rev
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=green
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind  7734..7762
    /label=garz-7193-F
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
rep_origin  complement(2247..2929)
```

```

    /label=ColE1 origin
    /ApEinfo_fwdcolor=gray50
    /ApEinfo_revcolor=gray50
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
CDS      7488..7494
    /label=New Feature(3)
    /ApEinfo_label=New Feature
    /ApEinfo_fwdcolor=#99ccff
    /ApEinfo_revcolor=#99ccff
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
CDS      complement(4323..4391)
    /label=LacZ alpha
    /ApEinfo_fwdcolor=#6495ed
    /ApEinfo_revcolor=#6495ed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature 7228..7230
    /label=E740K
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_binding complement(1885..1907)
    /label=Lac0
    /ApEinfo_fwdcolor=#6495ed
    /ApEinfo_revcolor=#6495ed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind complement(6005..6027)
    /label=garz-JF01603-R
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
```

```
CDS      complement(3027..3686)
         /label=AmpR
         /ApEinfo_fwdcolor=yellow
         /ApEinfo_revcolor=yellow
         /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
         width 5 offset 0
misc_feature  5928..5928
         /label=New Feature(4)
         /ApEinfo_label=New Feature
         /ApEinfo_fwdcolor=#ffffcc
         /ApEinfo_revcolor=#00ff00
         /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
         width 5 offset 0
primer_bind  4436..4459
         /label=M13F
         /ApEinfo_fwdcolor=#0a00ff
         /ApEinfo_revcolor=#0a00ff
         /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
         width 5 offset 0
primer_bind  6243..6261
         /label=garz-GF4
         /ApEinfo_fwdcolor=#ff00bd
         /ApEinfo_revcolor=#ff0003
         /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
         width 5 offset 0
primer_bind  4462..4479
         /label=M13F_GW
         /ApEinfo_fwdcolor=#ff00bd
         /ApEinfo_revcolor=#ff0003
         /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
         width 5 offset 0
primer_bind  complement(1859..1879)
         /label=M13R_GW
         /ApEinfo_fwdcolor=#ff00bd
         /ApEinfo_revcolor=#ff0003
         /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
```



```
width 5 offset 0
primer_bind    3998..4015
               /label=pQE60-F
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature   complement(1350..1708)
               /label=Copia Promoter?
               /ApEinfo_fwdcolor=#cde7f7
               /ApEinfo_revcolor=#cde7f7
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature   4892..4909
               /label=Metallothionein_primer
               /ApEinfo_fwdcolor=#ff3600
               /ApEinfo_revcolor=#ff3600
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature   10974..10991
               /label=6xHis
               /ApEinfo_fwdcolor=cyan
               /ApEinfo_revcolor=#00ff00
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
CDS            5014..6867
               /label=garz-PB(3)
               /ApEinfo_label=garz-PB
               /ApEinfo_fwdcolor=#99ccff
               /ApEinfo_revcolor=#cde7f7
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind    9076..9093
               /label=garz-GF9
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
```

```
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    8518..8537
    /label=garz-GF8
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    6868..6888
    /label=garz-GF5
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    7419..7436
    /label=garz-GF6
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature   7495..7714
    /label=Phosphatidylinositol-phosphate binding(1)
    /ApEinfo_label=Phosphatidylinositol-phosphate binding
    /ApEinfo_fwdcolor=#ff81f0
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    7921..7940
    /label=garz-GF7
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
CDS            7715..8945
    /label=Phosphatidylinositol-phosphate binding(2)
    /ApEinfo_label=Phosphatidylinositol-phosphate binding
```

```
    /ApEinfo_fwdcolor=#ff81f0
    /ApEinfo_revcolor=#99ccff
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    5120..5139
    /label=garz-GF3
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
CDS            6868..6888
    /label=New Feature(5)
    /ApEinfo_label=New Feature
    /ApEinfo_fwdcolor=#99ccff
    /ApEinfo_revcolor=#99ccff
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
CDS            8946..8982
    /label=New Feature(6)
    /ApEinfo_label=New Feature
    /ApEinfo_fwdcolor=#99ccff
    /ApEinfo_revcolor=#99ccff
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    9715..9736
    /label=garz-GF10
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature   7279..7487
    /label=Sec7 domain
    /ApEinfo_fwdcolor=#0080ff
    /ApEinfo_revcolor=#2098da
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
```

```
misc_feature    7333..7335
                /label=191A
                /ApEinfo_fwdcolor=cyan
                /ApEinfo_revcolor=#00ff00
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
                width 5 offset 0
misc_feature    7488..7494
                /label=Sec7 domain(1)
                /ApEinfo_label=Sec7 domain
                /ApEinfo_fwdcolor=#0080ff
                /ApEinfo_revcolor=#00ff00
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
                width 5 offset 0
primer_bind     5731..5749
                /label=garz-GF12
                /ApEinfo_fwdcolor=#ff00bd
                /ApEinfo_revcolor=#ff0003
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
                width 5 offset 0
CDS             7495..7714
                /label=New Feature(7)
                /ApEinfo_label=New Feature
                /ApEinfo_fwdcolor=#99ccff
                /ApEinfo_revcolor=#99ccff
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
                width 5 offset 0
primer_bind     complement(8216..8241)
                /label=garz-7193-R
                /ApEinfo_fwdcolor=#ff00bd
                /ApEinfo_revcolor=#ff0003
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
                width 5 offset 0
misc_feature    6005..6027
                /label=Rab1 binding
                /ApEinfo_fwdcolor=#66ccff
                /ApEinfo_revcolor=#00ff00
```

```
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
misc_feature    5936..5936
        /label=New Feature(8)
        /ApEinfo_label=New Feature
        /ApEinfo_fwdcolor=#ffffcc
        /ApEinfo_revcolor=#00ff00
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
misc_feature    complement(225..1250)
        /label=Hyg
        /ApEinfo_fwdcolor=#ffffcc
        /ApEinfo_revcolor=#00ff00
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
misc_feature    1254..1268
        /label=New Feature(9)
        /ApEinfo_label=New Feature
        /ApEinfo_fwdcolor=cyan
        /ApEinfo_revcolor=#00ff00
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
gene            complement(143..173)
        /gene="puro (variant)"

/translation="MGSCAPFGRALRVVGRASGTGLAGHAPGARSFGHLDVGGDGEAE
PLVEGEVAGRGLQEGGHPGALGRLHSGEHDGAAQTLALVVGRDADGGQEPRGLLGPV
RRQEAFLLLLRGQPGTAQLGHARADLGEHRPRFDALRRGPDHRGAVVRDPHLADVEP
DAREEEFLQLGDPLDVAVRVDGVARGGVVGERGGEGAYGPGDVVAGGEAHRGLVLGHG
        RSSPCEGSGAWVRGWRHRSWRPTCRHASRYRIPAARGICCNL*"
        /label=puro (variant)
        /ApEinfo_fwdcolor=#ffffcc
        /ApEinfo_revcolor=#ffffcc
```

```
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}  
    width 5 offset 0  
misc_feature 95..115  
    /label=New Feature(10)  
    /ApEinfo_label=New Feature  
    /ApEinfo_fwdcolor=cyan  
    /ApEinfo_revcolor=#00ff00  
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}  
    width 5 offset 0
```

ORIGIN

```
1 ataagctgca ataaacaagt tctagagtcg gtgggcctcg ggggcgggtg cggggtcggc  
61 ggggccgccc cgggtggctt cggtcggagc catggggtcg tgcgctcctt tcggtcgggc  
121 gctgcgggtc gtggggcggg cgtcaggcac cgggcttgcg ggtcatgcac cagttgctgg  
181 ctgaggcggt ctcgaaatca gctcttgttc ggtcggcatc tactctattc ctttgcctc  
241 ggacgagtcg tggggcgtcg gtttccacta tcggcgagta cttctacaca gccatcggtc  
301 cagacggccc cgcttctgcg ggcgatttgt gtacgcccga cagtcccggc tccggatcgg  
361 acgattgcgt cgcacgcacc ctgcgcccga gctgcatcat cgaaattgcc gtcaaccaag  
421 ctctgataga gttggtcaag accaatgcgg agcatatacg cccggagccg cggcgatcct  
481 gcaagctccg gatgcctccg ctggaagtag cgcgtctgct gctccataca agccaaccac  
541 ggcctccaga agaagatggt ggcgacctcg tattgggaat ccccgaacat cgcctcgctc  
601 cagtcaatga ccgctgttat gcggccattg tccgtcagga cattgttggg gccgaaatcc  
661 gcgtgcacga ggtgccggac ttcggggcag tcctcggccc aaagcatcag ctcatcgaga  
721 gcctgcgcga cggacgcact gacggtgctg tccatcacag tttgccagtg atacacatgg  
781 ggatcagcaa tcgcgcatat gaaatcacgc catgtagtgt attgaccgat tccttgcggt  
841 ccgaatgggc cgaacccgct cgtctggcta agatcggccg cagcgatcgc atccatggcc  
901 tccgcgaccg gctgcagaac agcgggcagt tcggtttcag gcaggctctg caacgtgaca  
961 ccctgtgcac ggcgggagat gcaataggtc aggctctcgc tgaattcccc aatgtcaagc  
1021 acttccggaa tcgggagcgc ggccgatgca aagtgccgat aaacataacg atctttgtag  
1081 aaaccatcgg cgcagctatt taccgcagg acatatccac gccctctac atcgaagctg  
1141 aaagcacgag attcttcgcc ctccgagagc tgcacaggt cggagacgct gtcgaacttt  
1201 tcgatcagaa acttctcgac agacgtcgcg gtgagttcag gctttttcat ggtggtcgtc  
1261 tccttgtgag gggtcagggg cgtgggtcag gggatggtgg cggcaccggt cgtggcggcc  
1321 gacctgcagg catgcaagct atcgaattcc tgcagcccgg gggatctggt gtaatttata  
1381 atttatattt ctttcttaa taaataaata aatagtcaag tttatgttg agttttatga  
1441 tttatatttt aagttatttc aactgcaaca ccagcaccac gacctacta cagcaaaaaa  
1501 cgtacaagaa ggaaagaagg aataaaaaga gtggtattct cttacaatat gttttatggc
```

1561 ataaaaggtg tggccattca tatcaaatat aaagtagtgt tgtttaacgt tacttttgta  
1621 ggttgaatag tatattccaa cagatgatga ggggttccca atcctaaacc catttgccgt  
1681 tcccagaagc atgaaaccac cagcaccgg atcctctaga acaacaaca ttgcattcat  
1741 tttatgtttc aggttcaggg ggaggtgtgg gaggtttttt aaagcaagta aaacctctac  
1801 aatgttgga tggctgatta tgatcagtcg acctgcaggc atgcaagctt ggcgtaatca  
1861 tggcatagc tgtttcctgt gtgaaattgt tatccgctca caattccaca caacatacga  
1921 gccggaagca taaagtgtaa agcctggggg gcctaagag tgagetaact cacattaatt  
1981 gcgttgcgct cactgcccgc tttccagtcg ggaaacctgt cgtgccagct gcattaatga  
2041 atcggccaac gcgcggggag aggcggtttg cgtattggg gctcttccgc ttctcgctc  
2101 actgactcgc tgcgctcggc cgttcggctg cggcgagcgg tatcagctca ctcaaaggcg  
2161 gtaatacggc tatccacaga atcaggggat aacgcaggaa agaacatgtg agcaaaaggc  
2221 cagcaaaagg ccaggaaccg taaaaggcc gcgttgctgg cgtttttcca taggctccgc  
2281 cccctgacg agcatcaca aaatcgacgc tcaagtcaga ggtggcgaaa cccgacagga  
2341 ctataaagat accaggcgtt tccccctgga agctccctcg tgcgctctcc tgttccgacc  
2401 ctgccctta ccggatacct gtccgccttt ctcccttcgg gaagcgtggc gctttctcat  
2461 agctcacgct gtaggtatct cagttcgggt taggtcgttc gctccaagct gggctgtgtg  
2521 cacgaacccc ccgttcagcc cgaccgctgc gccttatccg gtaactatcg tcttgagtcc  
2581 aacccggtaa gacacgactt atcgccactg gcagcagcca ctggtaacag gattagcaga  
2641 gcgaggtatg taggcggtgc tacagagttc ttgaagtggg ggcctaacta cggctacact  
2701 agaagaacag tatttggtat ctgcgctctg ctgaagccag ttaccttcgg aaaaagagtt  
2761 ggtagctctt gatccggcaa acaaacacc gctggtagcg gtggtttttt tgtttgcaag  
2821 cagcagatta cgcgagaaa aaaaggatct caagaagatc ctttgatctt ttctacgggg  
2881 tctgacgctc agtgaacga aaactcacgt taagggattt tggcatgag attatcaaaa  
2941 aggatcttca cctagatcct tttaaattaa aatgaagt tttaatcaat ctaaagtata  
3001 tatgagtaaa cttggtctga cagttacca tgcttaatca gtgaggcacc tatctcagcg  
3061 atctgtctat ttcgttcatc catagttgcc tgactccccg tcgtgtagat aactacgata  
3121 cgggagggct taccatctgg cccagtgct gcaatgatac cgcgagacc acgctcaccg  
3181 gctccagatt tatcagcaat aaaccagcca gccggaaggg ccgagcgag aagtggcctt  
3241 gcaactttat ccgcctccat ccagtctatt aattgttgcc ggaagctag agtaagtagt  
3301 tcgccagtta atagtttgcg caacgttggt gccattgcta caggcatcgt ggtgtcacgc  
3361 tcgctgtttg gtatggcttc attcagctcc ggttcccaac gatcaaggcg agttacatga  
3421 tccccatgt tgtgcaaaaa agcggtagc tccttcggtc ctccgatcgt tgcagaagt  
3481 aagttggccg cagtgttatc actcatggtt atggcagcac tgcataattc tcttactgtc  
3541 atgccatccg taagatgctt ttctgtgact ggtgagtact caaccaagtc attctgagaa  
3601 tagtgtatgc ggcgaccgag ttgctcttgc ccggcgtcaa tacgggataa taccgcgcca  
3661 catagcagaa ctttaaaagt gctcatcatt ggaaaacgtt ctccggggcg aaaactctca

3721 aggatccttac cgctgttgag atccagttcg atgtaacca ctcgtgcacc caactgatct  
3781 tcagcatcctt ttactttcac cagcgtttct gggtgagcaa aaacaggaag gcaaaatgcc  
3841 gcaaaaaagg gaataagggc gacacggaaa tgttgaatac tcatactctt cttttttcaa  
3901 tattattgaa gcatttatca gggttattgt ctcatgagcg gatacatatt tgaatgtatt  
3961 tagaaaaata aacaaatagg ggttccgcgc acatttcccc gaaaagtgcc acctgacgtc  
4021 taagaaacca ttattatcat gacattaacc tataaaaaata ggcgtatcac gaggcccttt  
4081 cgtctcgcgc gtttcggtga tgacggtgaa aacctctgac acatgcagct cccggagacg  
4141 gtcacagctt gtctgtaagc ggatgccggg agcagacaag cccgtcaggg cgcgtcagcg  
4201 ggtgttggcg ggtgtcgggg ctggcttaac tatgcggcat cagagcagat tgtactgaga  
4261 gtgaccata tgcggtgta aataccgcac agatgcgtaa ggagaaaata ccgcatcagg  
4321 cgccattcgc cattcaggct gcgcaactgt tgggaagggc gatcgggtgcg ggcctcttcg  
4381 ctattacgcc agctggcgaa agggggatgt gctgcaaggc gattaagttg ggtaacgcc  
4441 gggttttccc agtcacgacg ttgtaaacg acggccagtg aattaattcg ttgcaggaca  
4501 ggatgtggtg cccgatgtga ctagctcttt gctgcaggcc gtcctatcct ctggttccga  
4561 taagagaccc agaactccgg cccccaccg cccaccgcc ccccataca tatgtggtac  
4621 gcaagtaaga gtgcctgcgc atgccccatg tgccccacca agagctttgc atcccataca  
4681 agtccccaaa gtggagaacc gaaccaattc ttcgcgggca gaacaaaagc ttctgcacac  
4741 gtctccactc gaatttggag ccggccggcg tgtgcaaaag aggtgaatcg aacgaaagac  
4801 ccgtgtgtaa agccgcgttt ccaaaatgta taaaaccgag agcatctggc caatgtgcat  
4861 cagttgtggt cagcagcaaa atcaagtga tcatctcagt gcaactaaag gggggatcta  
4921 gatcggggta ccgccacat gggtaagcct atccctaacc ctctcctcgg tctcgattct  
4981 acgactagtg gaggaggagg ttctggtggt ggtGCGCTTC CAGGCAACGG CATCTACGTG  
5041 GTGCGGGGCG AAATGGCCAC CCTGATGACG GCGATGCGAC GTGGAACGCG TTGGAATGCC  
5101 ACCGCCTACG TGGACGACGA GAATGACTCG CTGCTGAAGC TGTTATTGA CCTCAAGCAT  
5161 GAGCTAAATC GGATCGAGGA CCTGCGTCAG ATCGAGCCTC AGGTCTTCCT GGCTCCGTTT  
5221 CTTGAGGTGA TTCGCACGGC GGACGCCACG GGTCCGTTGA CTAGTCTAGC CTTGGCCTCG  
5281 GTTAACAAAT TATTGTCTTA CGGGTAATA GATCCCACGT CTCAAATCT GGCTGACATT  
5341 GTGAGCGCA TTGCCGATGC TGTGACACAT GCCCGCTTTA TGGGCACCGA TCAGTCCCTCG  
5401 GATGGTGTC CTTTATGCG AGTGATTGAG GTGCTGCACA CGTTATCCG CAGTCCCGAA  
5461 GGAGCCGCCG TCAGCAATGT GTCCATGTGC GAGGTGATGC TCAGCTGCTT CAAGATCTCC  
5521 TTCGAGCCGA GGCTGAGTGA ACTGCTTCGT CGCTCGGCGG AAAAATCGCT CAAGGACATG  
5581 GTGCTGCTCT TCTTCATGCG CCTTCCCCAG TTCGCCGAAG AGCGAAGTGA CACCATGCTC  
5641 CAGAAGCGAT TTAATATCGG CGATGCTGCC AGCGGAGCTA CCCAAGAAAA ACTAAAGCGT  
5701 AAGACGGTTG CCCAAGCCCA GACAGCACCC AGGAAATCGT CAGCAGTGGG GGAACCGCCT  
5761 CAAACACCGC AGTCTGCCAA CCTGACGGTG CCAGGGCACT TGAAGGCACC CATATTGGCC  
5821 ACCACACCCG CCAGTCCAGC GGGAAACATA TTGACATGC AGGGCAAGAT TACGCAGACA



5881 CCAACAACAA CGGCGAGCAC GGGGAAGAT GAAACCACTG TCCCGGAGAC TCCTGTTATT  
5941 CAAGTGGAGT CGACTGAATC GGAGCCCTTG CTGGACGGCG AAACGGGTGA AGCAACCAGC  
6001 ACCCTGGCCG AGGCAAATAG TAGCGAGTAC ATCAACTCGG TGGGCGTCCG TTTTACACAG  
6061 CAGTCCACCG ATCACGATGT AACATCACTA TCCCCTTATG GCCTGCCCTT CATCCAGGAG  
6121 TTGTTCCGAT TCCTCATAAT CCTCTGCAAT CCTCTGGATA AGCAGAACTC GGATAGCATG  
6181 ATGCACACGG GTCTTAGTCT ACTTACTGTA GCTTTTGAAG TCGCAGCCGA TAATATTGGA  
6241 AAGTATGAGG GCTTGCTGGA GCTGGTTAAG GACGACTTAT GCAGAACTT AATATCGCTT  
6301 CTCAGCTCAG AGCGGCTTAG CATCTTTGCC GCCGATTTGC AGCTCTGTTT TTTGCTTTTC  
6361 GAGTCTCTCC GCGGACATCT CAAGTTTCAG CTGGAAGCCT ACCTTAGAAA ATTGAGCGAG  
6421 ATTATTGCTA GCGATAATCC CAAGACGCCC TACGAAATGC GAGAACTCGC TCTGGACAAT  
6481 CTAAGTGCAGT TGTGGCGCAT TCCCGGCTTC GTCACGGAAT TGTATATCAA CTACGATTGT  
6541 GACTTGTACT GCACGGATAT GTTTGAAGT TTAGACAAACC TACTGAGCAA GTATACGCTG  
6601 TCAGCAACGA ATGCAGTTTA TAGCACCCAC ATTATCTCAA TGGACACCCT GTTAAGTGTG  
6661 ATAGACAGTA TCGAGCGAAA TTGTGCCGCG AGCAAGAATA GCAGCAACAA CAGAGAGTCC  
6721 TTGCCAGAAG CTGCCCCAGC AACAGGTGGC AGCCGCCATT CTCGCCACAA CAGCGGATTG  
6781 GAGGGAATCG TAATTGATTC TGGCAATAGT GTAGCTGCAG AAGAGAAAAGT GGAGAACATC  
6841 GCAAGCTTTA TAAATGCGAG CTCACATCGA CTACGACTAC AATCTGGCGG AGAGGGAGTG  
6901 GGAATAACCA GTGAACAGCT GGCCAAGTGC AAACAGAAGA AGCGTCTGCT ATCCCAAGGC  
6961 ACAGAGCGAT TTAATCAGCG TCCAGAGAAA GGAATCCAGT ATCTGCAAGA ACACGGCATC  
7021 CTAATGCCC AGCTTGATCC CATGCAGGTG GCCCTGTTCC TTCGAGAAAA TCCCGGGCTC  
7081 GATAAAAAAA TGATTGGCGA ATATATCTCG AAAAAGAAAA ACGTCGACTC TAAGATTCTA  
7141 ATTAATTTTG TGGACTCGTT TGATTTCACT GGTCTTCGAG TGGATCAAGC ATTGCGTCTT  
7201 TATCTGGAGA CCTTCAGATT GCCCGGAGAG GCTCCATTGA TCTTTTTGTT GCTGGAACAC  
7261 TTTTCTGATC ATTGGCATAA ACAAACCAA GATCCGTTTG CCAACGTAGA CGCTGCTTTT  
7321 CGCTTGGCCT ATGCCATCAT CATGCTGAAC ATGGATCAGC ACAACTCGAA CGCGAAGCGT  
7381 TTAAATGTTC CAATGACGCT CGAGGACTTC ACTAAGAATT TGCGTGGTCT AAACGGTGGC  
7441 GAAGATTTTC ATCAAGAAAT GCTGGCTCAA GTCTTTAATG CAATCAAGAA CGAAGAGATC  
7501 GTTATGCCAG CAGAGCAAAC GGGTCTGGTG CGTGAAAATT ATCAATGGAA AGTACTGCTT  
7561 CGACGAGGAG ACACGCACGA TGGACATTTT CACTATGTGC ATGACGCATC ATACGACGTG  
7621 GAGATCTTCA ATATTGTGTG GGGTGCTTCT CTGAGCGCCC TAAGCTTTAT GTTTGATAAA  
7681 AGCACTGAAA CGGGCTACCA AAGAACTCTA GCAGGTTTCA GCAAATCCGC TGCCATATCG  
7741 GCGCACTATA ATCTGCATTC GGACTTCGAT GCCCTCGTTT TAACTCTCTG CAAATTCACA  
7801 ACGCTGCTGA GCAGCGTAGA ACAGCATGAG CCCGCTCCGG CGAACAATGA AACCCAGCAA  
7861 GCTGTGAACT TTGATTGAA CGGAAAGGCT CAGGCTGCCA TGCGAACGGT GTTTCTATTG  
7921 GTTCACGACT ACGGCGATTG CTTAAGAGAG AGCTGGAAAC ACATTTTGGG CCTATATCTG  
7981 CAGCTTTTCC GTCTAAAGTT GCTGCCAAAA TCATTGATCG AAGTGAAGA CTTTTGTGAG

8041 GCGAACGGAA AGGCCATGTT AATCCTGGAA AAGCCCCGCG AGAAGCAGGA ATCGGGACTA  
8101 TTTTCCAGCC TGACTCATT TATCAGCTCG GAGGGTCAGC GAGAACCAAC GTACGAGGAG  
8161 CAGGACTTCA TCAAAGTGGG ACGGAAGTGC ATTAAGGAGT GCCAGCTGGA TCAAATGCTG  
8221 CAGGAATCAA AGTTTGTGCA ACTAGAGTGC CTGCAGGAGT TGCTTAAATG CGTTCTAGCG  
8281 CTAAGTGAAG CTCCTCAGGG GCACAAATCC ATTGGCCTGC CGTACGCCGA AGATCAAACCT  
8341 GTTTTCTGGA TGGAATTTTT GGTCAAGATA GTTGTTCATA ACCGGGATCG CATGATACCG  
8401 CTGTGGCCAG CAGTTCGAGA CCAAATGTAC CTAAGTCTTA TGGGCAGTGC CTCCTGTGGA  
8461 TACGACTACC TACTCAACCG ATGCATTGTA GCGGTCTTAA AACTAGCTAT CTATCTGATG  
8521 CGAAACGAAG AACTGTGTCC GATCGTATTG CAATCGCTCA AGATGCTTTT AATGCTTAAG  
8581 CCAGCCTTGT TGCTGCGCAT TTCTAAACAG ATTTCCATTG GTATCTATGA GCTGCTCAAG  
8641 ACGTCGGCCC AAAATATTCA TTCCGAGCAG GACTGGCAGA TTATTTTCAA TCTACTTGAA  
8701 TCGTGGGAG CCGGTGCTGT GCCGCCAAT TATGATGATG CCCAGCTGCC ATTGCCGCC  
8761 AACGGAAGTG CAAAGTCTGA TGGCGCTATA AGTGGCGAAG AGGACGCAAC TGCCGTGCCA  
8821 GAGCGTGGTT ACACCTCGGA TTCGGAGATC ACGAAAGCAT CTGCAGCACC TGCAGTCTCC  
8881 AGTCCAAGTG CTGAGAAGTCT GATTCTGGTC AATAACAAGG ACAGTGAATT GACTACGGCC  
8941 TCTAGACCAC AATCTCCGCC TAGCCTGAGT GCTCCTCCAG TAAATACGCT TGTGTACAAT  
9001 TGCCAGCTAC TAGACCACGC TCCGTTTGCT CTTTTCAAGT GCTGGGATTC GCTGGCGTTT  
9061 ATCGTGCACA GTGTGGCACA CATCACGCCT TACAATTTG AAGCCTGCGT TCGCTGCATC  
9121 CGCATCTTTG TGGAGGCTTG TCGGGATGGA GGTATACGCC AGCGGCGAAA GCTGGAATCG  
9181 GCGGCTAAGC AGAAAAGTTC CAAGAAGCGC AGCGAACGCA AACCAGGCGT GGCTTCTCTC  
9241 GCCTCGAGTA GTAATCTTAC TCTTCTGACG GGCAGCCCGT CCGACAACCA GATAAACGGA  
9301 AATGCGGCAG AGCAGGAGGA CCTGGCCAG CGCTACGAAC AGTTGTCCAT TCAACTGCTG  
9361 GACCTGATGT ATACGTTGTA CACGCGAACT GCCCAAATCT TCCGATGGTG GGCAGGAAGAA  
9421 GGATGCACAG TGCCGAGTC GGCAGCTTTG TGGTCACCGG GCTGGTGTCC ATTGCTTCTCAG  
9481 GGAATCGCCA GGCTGGCAAT GGATCGACGG CGAGAGGTGC GCACCCATGC CATATCGTGC  
9541 CTGCAGCAGC GGGCATTGCT AGTCCATGAC CTGCAAACGT TGTCGGGAAC GGAGTGGTGC  
9601 TCTTGCTTCC ACCAGGTGCT GTTCCCCCTC CTAAACGAAC TGCTGCCCGA GAGTAATGCA  
9661 GCCGCGCAAC TGATGCGCG TCTCCTCGAA GAGTCGCGTA TACGAACGGC CACCATTATG  
9721 TCTAAGGTGT TCCTGCAACA CCTGACCAGC CTCATCGAGC TGGGAAATGC TTTTAACGAG  
9781 CTGTGGCTGG ATATATTGGA CTACATTGAG CGCTTTATGA AGGTGGGATC GGACACATTG  
9841 TCCGAGCAGA TGCAGGAGAT ACTGAAGAAC ATGCTGCTGG TGATGCATTC AGTGCAGTGC  
9901 TTCCACAATC AGGATGGTAG TTTACAGCAG GCTCTTTGGG AGCTAACCTG GCGACGCATC  
9961 GGCGAATTTT TGCCCAACCT GAAGGAGGAG CTTTTCCACG ACGAAGGCAA GCGAGCTCAG  
10021 ACCTTAACGA ACCCAGCTCC ACAGGCAGCT GTGGCTGCCG CTCCACAGCA ACAGTTACCA  
10081 GCGGTGACCA TTTTGCCAG GCAAACCCAG GTTTCCAACG AGTTAGTGGT GAGCGCGCCT  
10141 ACTCCGCCGG CAGCCACACC TTTGCTGGGC TCTCCCGTGC AGTCGCCGAG GCGGAGCATA

<p>10201 A T A C T G C A G C C A C C C A T G G C G A T G T A C T G C A A C A G C C C G C C A G C T T T G T A T T G C T C A G  10261 C C C A T T A T T G T G C C A C C C C A G C C G C T G C A G T T A C G G A C C C A A T A C C A C C A A G T A C A T T A  10321 T T G C C G G A T T T G G T G A A T G A G G C A A C T G C T G C T G C C G T G C A A G C C A C G A C C A C G T C C C C G  10381 A C G C A C A G C C C G C A G G A G G C G G A G C A G C C G C T T C A A T A G T G C A G C A G A C C A A C A T C G T A  10441 A C C A C C A A C A A T A C G T A C A A T A G C T A C G C C A T T G A G G T G C C C A T G G C G C C G G A G A C A A C T  10501 G C G G A A C A G T T T G G G C A G C A G C A G C A A C T G C T T T A C C A A C A G T A C T A T C A A C A G T A T  10561 C A G C C C C A G C A G C A A C A G T T G C C G G C T C C A G C C A G C G A C C C T G C C A T C A A T G T G C C A A T T  10621 A G T C A T C T G C T G G C C G A A A T G C G T A C C C C T C G C T C C C C A A A T G C C G C A G G C A T C C A T T  10681 G T G C A C A G C T T T G C G C C C G T T T A C G A A A G C C A G G C G G C G A C G A G T G G A G C T G G G A C A G C A  10741 G C A G C G G A C A T C T A T C A G G A G T A T G T G C A A A A T C C G T A C A C C T T A C G T T G C A A C A G C A T  10801 C C C C A A C A G C A G C T C C A T C A G C A G C A G C A G C A C A C A A C A G C A G G C T A C A G G A A T G G C C  10861 A A C G C A T T T C C G C C G T T G C C A G C C A G C C A A C T A C T T T A A T G T G A A T G T G G A C C C C A G T  10921 A G C A T A C C G C C G G A T C G G A A C T G C T C T A C G G C C A G C A G T A A c g c g t a c c g g t c a t c a t c  10981 a c c a t c a c c a t t g a g t t t a a a c c c g t g a t c a g c c t c g a c t g t g c c t t c t a a g a t c c a g a  11041 c a t g a t a a g a t a c a t t g a t g a g t t t g g a c a a c c a c a a c t a g a a t g c a g t g a a a a a a a t g  11101 c t t t a t t t g t g a a a t t t g t g a t g c t a t t g c t t t a t t t g t a a c c a t t</p> <p>//</p>					
<p>pMT-hyg-V5::garz<sup>E740K</sup></p>					
LOCUS	pMT-hyg-V5::garzE740K	11146 bp	ds-DNA	circular	28-FEB-2020
DEFINITION	pMT-puro Sequencing Result				
ORGANISM	other sequences; artificial sequences; vectors.				
COMMENT	pMT-puro from 1 to 4724				
COMMENT	ApEinfo:methylated:1				
FEATURES	Location/Qualifiers				
misc_feature	complement(11057..11076)				
	/label=EBV_rev_primer				
	/ApEinfo_fwdcolor=#ff3600				
	/ApEinfo_revcolor=#ff3600				
	/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}				
	width 5 offset 0				
CDS	6913..7278				
	/label=New Feature				
	/ApEinfo_fwdcolor=#99ccff				
	/ApEinfo_revcolor=#99ccff				
	/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}				

```
width 5 offset 0
misc_feature 4942..4983
  /label=V5
  /ApEinfo_fwdcolor=#ffffcc
  /ApEinfo_revcolor=#ffffcc
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature complement(11014..11031)
  /label=BGH_rev_primer
  /ApEinfo_fwdcolor=#ff3600
  /ApEinfo_revcolor=#ff3600
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature complement(4327..4482)
  /label=lacZ_a
  /ApEinfo_fwdcolor=#ff3600
  /ApEinfo_revcolor=#ff3600
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
CDS 9094..10959
  /label=garz-PB
  /ApEinfo_fwdcolor=#99ccff
  /ApEinfo_revcolor=#cde7f7
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 10963..10964
  /label=V5(1)
  /ApEinfo_label=V5
  /ApEinfo_fwdcolor=cyan
  /ApEinfo_revcolor=#00ff00
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 7330..7332
  /label=190Y
  /ApEinfo_fwdcolor=#ccff66
  /ApEinfo_revcolor=#00ff00
```

```
promoter      /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
              width 5 offset 0
              4463..4479
              /label=M13_forward20_primer
              /ApEinfo_fwdcolor=#ccffed
              /ApEinfo_revcolor=#ccffed
              /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
              width 5 offset 0
CDS           5560..5586
              /label=New Feature(1)
              /ApEinfo_label=New Feature
              /ApEinfo_fwdcolor=#99ccff
              /ApEinfo_revcolor=#99ccff
              /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
              width 5 offset 0
misc_feature  4448..4470
              /label=M13_pUC_fwd_primer
              /ApEinfo_fwdcolor=#ff3600
              /ApEinfo_revcolor=#ff3600
              /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
              width 5 offset 0
primer_bind   5560..5586
              /label=JF01603-F
              /ApEinfo_fwdcolor=#ff00bd
              /ApEinfo_revcolor=#ff0003
              /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
              width 5 offset 0
misc_feature  complement(4113..4135)
              /label=pGEX_3_primer
              /ApEinfo_fwdcolor=#ff3600
              /ApEinfo_revcolor=#ff3600
              /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
              width 5 offset 0
misc_feature  8946..8982
              /label=Phosphatidylinositol-phosphate binding
              /ApEinfo_fwdcolor=#ff81f0
```

```
promoter      /ApEinfo_revcolor=#00ff00
              /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
              width 5 offset 0
              complement(3926..3954)
              /label=AmpR_promoter
              /ApEinfo_fwdcolor=#ccffed
              /ApEinfo_revcolor=#ccffed
              /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
              width 5 offset 0
gene          complement(3024..3884)
              /gene="Ampicillin"
              /label=Ampicillin
              /ApEinfo_fwdcolor=#ffffcc
              /ApEinfo_revcolor=#ffffcc
              /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
              width 5 offset 0
CDS          8983..9075
              /label=garz-PB(1)
              /ApEinfo_label=garz-PB
              /ApEinfo_fwdcolor=#99ccff
              /ApEinfo_revcolor=#cde7f7
              /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
              width 5 offset 0
rep_origin    complement(2250..2869)

/translation="MSIQHFRVALIPFFAAFLPVFAHPETLVKVKDAEDQLGARVGY
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHVTRL
DRWEPELNEAIPNDERDTMPVAMATTLRKLTTGELLTLASRQQLIDWMEADKVAGPL
LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
EIGASLIKHW*"
              /label=pBR322_origin
```

```

    /ApEinfo_fwdcolor=pink
    /ApEinfo_revcolor=pink
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
CDS      9076..9093
    /label=New Feature(2)
    /ApEinfo_label=New Feature
    /ApEinfo_fwdcolor=#99ccff
    /ApEinfo_revcolor=#99ccff
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
promoter complement(1912..1941)

/translation="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGY
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHVTRL
DRWEPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPL
LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
    EIGASLIKHW*"
    /label=lac_promoter
    /ApEinfo_fwdcolor=#ccffed
    /ApEinfo_revcolor=#ccffed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature 4990..5013
    /label=Linker GL3
    /ApEinfo_fwdcolor=#ffcc66
    /ApEinfo_revcolor=#ffcc66
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature 4984..4989
    /label=SpeI
```

```

    /ApEinfo_fwdcolor=#ccff66
    /ApEinfo_revcolor=#ffffcc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature    8369..8370
    /label=MB05159
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature    complement(1876..1898)

/translation="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGY
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHVTRL
DRWEPELNEAIPNDERDTMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPL
LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
    EIGASLIKHW*"
    /label=M13_pUC_rev_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
promoter        complement(1859..1877)

/translation="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGY
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHVTRL
DRWEPELNEAIPNDERDTMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPL
```



```
LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
    EIGASLIKHW*"
    /label=M13_reverse_primer
    /ApEinfo_fwdcolor=#ccffed
    /ApEinfo_revcolor=#ccffed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    4489..4856
    /label=MT-promoter
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
CDS            6889..6912
    /label=garz-PB(2)
    /ApEinfo_label=garz-PB
    /ApEinfo_fwdcolor=#99ccff
    /ApEinfo_revcolor=#cde7f7
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    4462..4479
    /label=M13-fwd
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=green
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    10333..10351
    /label=garz-GF11
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    complement(1859..1879)
    /label=M13-rev
    /ApEinfo_fwdcolor=cyan
```

```
    /ApEinfo_revcolor=green
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    7734..7762
    /label=garz-7193-F
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
rep_origin    complement(2247..2929)
    /label=ColE1 origin
    /ApEinfo_fwdcolor=gray50
    /ApEinfo_revcolor=gray50
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
CDS
    7488..7494
    /label=New Feature(3)
    /ApEinfo_label=New Feature
    /ApEinfo_fwdcolor=#99ccff
    /ApEinfo_revcolor=#99ccff
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
CDS
    complement(4323..4391)
    /label=LacZ alpha
    /ApEinfo_fwdcolor=#6495ed
    /ApEinfo_revcolor=#6495ed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    7228..7230
    /label=E740K
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_binding    complement(1885..1907)
    /label=Lac0
```

```
    /ApEinfo_fwdcolor=#6495ed
    /ApEinfo_revcolor=#6495ed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    complement(6005..6027)
    /label=garz-JF01603-R
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
CDS            complement(3027..3686)
    /label=AmpR
    /ApEinfo_fwdcolor=yellow
    /ApEinfo_revcolor=yellow
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature   5928..5928
    /label=New Feature(4)
    /ApEinfo_label=New Feature
    /ApEinfo_fwdcolor=#ffffcc
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    4436..4459
    /label=M13F
    /ApEinfo_fwdcolor=#0a00ff
    /ApEinfo_revcolor=#0a00ff
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    6243..6261
    /label=garz-GF4
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    4462..4479
```

```

    /label=M13F_GW
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    complement(1859..1879)
    /label=M13R_GW
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    3998..4015
    /label=pQE60-F
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature    complement(1350..1708)
    /label=Copia Promoter?
    /ApEinfo_fwdcolor=#cde7f7
    /ApEinfo_revcolor=#cde7f7
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature    4892..4909
    /label=Metallothionein_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature    10974..10991
    /label=6xHis
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature    complement(225..1250)
```

```

        /label=Hyg
        /ApEinfo_fwdcolor=#ffffcc
        /ApEinfo_revcolor=#00ff00
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
misc_feature    1254..1268
        /label=New Feature(5)
        /ApEinfo_label=New Feature
        /ApEinfo_fwdcolor=cyan
        /ApEinfo_revcolor=#00ff00
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
gene            complement(143..173)
        /gene="puro (variant)"

/translation="MGSCAPFGRALRVVGRASGTGLAGHAPGARSFGHLDVGGDGEAE

PLVEGEVAGRGGQLQEGGHPGALGRLHSGEHDGAAQTLALVVRDADGGQEPRGLLGPV

RRQEA FHLLLRGQP GTAQLGHARADLGEHRPRFDALRRGPDRHRGAVVRDPHLADVEP

DAREEEFLQLGDPLDVAVRVDGVARGGVVGERGGEGAYGPGDVVAGGEAHRGLVLGHG
        RSPCEGSGAWVRGWRRHSWRPTCRHASYPARAARGICCNL*"
        /label=puro (variant)
        /ApEinfo_fwdcolor=#ffffcc
        /ApEinfo_revcolor=#ffffcc
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
misc_feature    95..115
        /label=New Feature(6)
        /ApEinfo_label=New Feature
        /ApEinfo_fwdcolor=cyan
        /ApEinfo_revcolor=#00ff00
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
CDS            5014..6867
```

```
    /label=garz-PB(3)
    /ApEinfo_label=garz-PB
    /ApEinfo_fwdcolor=#99ccff
    /ApEinfo_revcolor=#cde7f7
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    9076..9093
    /label=garz-GF9
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    8518..8537
    /label=garz-GF8
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    6868..6888
    /label=garz-GF5
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    7419..7436
    /label=garz-GF6
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature    7495..7714
    /label=Phosphatidylinositol-phosphate binding(1)
    /ApEinfo_label=Phosphatidylinositol-phosphate binding
    /ApEinfo_fwdcolor=#ff81f0
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
```

```
width 5 offset 0
primer_bind    7921..7940
               /label=garz-GF7
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
CDS            7715..8945
               /label=Phosphatidylinositol-phosphate binding(2)
               /ApEinfo_label=Phosphatidylinositol-phosphate binding
               /ApEinfo_fwdcolor=#ff81f0
               /ApEinfo_revcolor=#99ccff
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    5120..5139
               /label=garz-GF3
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
CDS            6868..6888
               /label=New Feature(7)
               /ApEinfo_label=New Feature
               /ApEinfo_fwdcolor=#99ccff
               /ApEinfo_revcolor=#99ccff
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
CDS            8946..8982
               /label=New Feature(8)
               /ApEinfo_label=New Feature
               /ApEinfo_fwdcolor=#99ccff
               /ApEinfo_revcolor=#99ccff
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    9715..9736
               /label=garz-GF10
```

```

/ApEinfo_fwdcolor=#ff00bd
/ApEinfo_revcolor=#ff0003
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 7279..7487
/label=Sec7 domain
/ApEinfo_fwdcolor=#0080ff
/ApEinfo_revcolor=#2098da
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 7333..7335
/label=191A
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 7488..7494
/label=Sec7 domain(1)
/ApEinfo_label=Sec7 domain
/ApEinfo_fwdcolor=#0080ff
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
CDS 6913..7278
/label=Sec7 domain
EQLAKVKQKKRLLSQGTERFNQRPEKGIQYLQEHGILNAELD
/ApEinfo_fwdcolor=#0080ff
/ApEinfo_revcolor=#99ccff
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind 5731..5749
/label=garz-GF12
/ApEinfo_fwdcolor=#ff00bd
/ApEinfo_revcolor=#ff0003
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
```



```
CDS          7495..7714
              /label=New Feature(9)
              /ApEinfo_label=New Feature
              /ApEinfo_fwdcolor=#99ccff
              /ApEinfo_revcolor=#99ccff
              /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
              width 5 offset 0
primer_bind   complement(8216..8241)
              /label=garz-7193-R
              /ApEinfo_fwdcolor=#ff00bd
              /ApEinfo_revcolor=#ff0003
              /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
              width 5 offset 0
misc_feature  6005..6027
              /label=Rab1 binding
              /ApEinfo_fwdcolor=#66ccff
              /ApEinfo_revcolor=#00ff00
              /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
              width 5 offset 0
misc_feature  5936..5936
              /label=New Feature(10)
              /ApEinfo_label=New Feature
              /ApEinfo_fwdcolor=#ffffcc
              /ApEinfo_revcolor=#00ff00
              /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
              width 5 offset 0
```

ORIGIN

```
1 ataagctgca ataacaaagt tctagagtcg gtgggcctcg ggggcgggtg cggggtcggc
61 ggggccgcc cgggtggctt cggtcggagc catggggtcg tgcgctcctt tcggtcgggc
121 gctgcgggtc gtggggcggg cgtcaggcac cgggcttgcg ggtcatgcac cagttgctgg
181 ctgaggcggt ctcgaaatca gctcttgttc ggtcggcatc tactctattc ctttgcctc
241 ggacgagtg cggggcgctg gtttccacta tcggcgagta cttctacaca gccatcggtc
301 cagacggccg cgcttctgcy ggcgatttgt gtacgcccga cagtcccggc tccggatcgg
361 acgattgcgt cgcacgacc ctgcgcccga gctgcatcat cgaaattgcc gtcaaccaag
421 ctctgataga gttggtcaag accaatgcgg agcatatacg cccggagccg cggcgatcct
481 gcaagctccg gatgcctccg ctcgaagtag cgcgtctgct gctccataca agccaaccac
```

541 ggctccaga agaagatggt ggcgacctg tattgggaat ccccgaacat cgctcgctc  
601 cagtcaatga ccgctgttat gcggccattg tccgtcagga cattgttggg gccgaaatcc  
661 gcgtgcacga ggtgccggac ttcggggcag tcctcggccc aaagcatcag ctcatcgaga  
721 gcctgcgcga cggacgcact gacggtgtcg tccatcacag tttgccagtg atacacatgg  
781 ggatcagcaa tcgcgcatat gaaatcacgc catgtagtgt attgaccgat tccttgcggt  
841 ccgaatgggc cgaacccgct cgtctggcta agatcggccg cagcgatcgc atccatggcc  
901 tccgcgaccg gctgcagaac agcgggcagt tcggtttcag gcaggctctg caacgtgaca  
961 ccctgtgcac ggcgggagat gcaataggtc aggtctctgc tgaattcccc aatgtcaagc  
1021 acttccggaa tcgggagcgc ggccgatgca aagtgccgat aaacataacg atctttgtag  
1081 aaaccatcgg cgcagctatt taccgcagg acatatccac gccctcctac atcgaagctg  
1141 aaagcacgag attcttcgcc ctccgagagc tgcacaggt cggagacgct gtcgaacttt  
1201 tcgatcagaa acttctcgac agacgtcgcg gtgagttcag gctttttcat ggtggtcgtc  
1261 tccttgtgag gggtcagggg cgtgggtcag gggatggtgg cggcaccggt cgtggcggcc  
1321 gacctgcagg catgcaagct atcgaattcc tgcagcccgg gggatctgtt gtaatttata  
1381 atttatattt ctttcttaa taaataaata aatagtcaag tttatgtttg agttttatga  
1441 tttatatttt aagttatttc aactgcaaca ccagcaccac gacctactta cagcaaaaaa  
1501 cgtacaagaa ggaagaagg aataaaaaga gtggtattct cttacaatat gttttatggc  
1561 ataaaagggt tggccattca tatcaaatat aaagtagtgt tgtttaacgt tacttttcta  
1621 ggttgaatag tatattccaa cagatgatga ggggttccca atcctaacc catttgccgt  
1681 tcccagaagc atgaaaccac cacgcaccgg atcctctaga acaacaacaa ttgcattcat  
1741 tttatgtttc aggttcaggg ggaggtgtgg gaggtttttt aaagcaagta aaacctctac  
1801 aatgttggtg tggctgatta tgatcagtcg acctgcaggc atgcaagctt ggcgtaatca  
1861 tggcatagc tgtttcctgt gtgaaattgt tatccgctca caattccaca caacatacga  
1921 gccggaagca taaagtgtaa agcctggggg gcctaagtag tgagctaact cacattaatt  
1981 gcgttgcgct cactgcccgc tttccagtcg ggaacctgt cgtgccagct gcattaatga  
2041 atcggccaac gcgcggggag aggcggtttg cgtattgggc gctcttcgc ttctcgctc  
2101 actgactcgc tgcgctcggg cgttcggctg cggcgagcgg tatcagctca ctcaaaggcg  
2161 gtaatacggg tatccacaga atcaggggat aacgcaggaa agaacatgtg agcaaaaggc  
2221 cagcaaaagg ccaggaaccg taaaaggcc gcggtgctgg cgtttttcca taggctccgc  
2281 cccctgacg agcatcaca aaatcgacgc tcaagtcaga ggtggcgaaa cccgacagga  
2341 ctataaagat accaggcgtt tcccctgga agctccctcg tgcgctctcc tgttccgacc  
2401 ctgccgctta ccggatacct gtccgccttt ctcccttcgg gaagcgtggc gctttctcat  
2461 agctcacgct gtaggtatct cagttcgggt taggtcgttc gctccaagct gggctgtgtg  
2521 cacgaacccc ccgttcagcc cgaccgctgc gccttatccg gtaactatcg tcttgagtcc  
2581 aacccggtaa gacacgactt atcgccactg gcagcagcca ctggtaacag gattagcaga  
2641 gcgaggatg taggcggtgc tacagagttc ttgaagtggt ggcctaacta cggctacact

2701 agaagaacag tatttggtat ctgcgctctg ctgaagccag ttaccttcgg aaaaagagtt  
2761 ggtagctctt gatccggcaa acaaacacc gctggtagcg gtggtttttt tgtttgcaag  
2821 cagcagatta cgcgagaaa aaaaggatct caagaagatc ctttgatctt ttctacgggg  
2881 tctgacgctc agtggaacga aaactcacgt taagggattt tggatcatgag attatcaaaa  
2941 aggatcttca cctagatcct tttaaattaa aatgaagtt ttaaatcaat ctaaagtata  
3001 tatgagtaaa cttggtctga cagttaccaa tgcttaatca gtgaggcacc tatctcagcg  
3061 atctgtctat ttcgttcatc catagttgcc tgactccccg tcgtgtagat aactacgata  
3121 cgggagggct taccatctgg cccagtgct gcaatgatac cgcgagacc acgctcaccg  
3181 gctccagatt tatcagcaat aaaccagcca gccggaagg ccgagcgag aagtggctct  
3241 gcaactttat ccgctccat ccagtctatt aattggtgcc ggaagctag agtaagtagt  
3301 tcgccagtta atagtttgcg caacgttggt gccattgcta caggcatcgt ggtgtcacgc  
3361 tcgtcgtttg gtatggcttc attcagctcc ggttcccaac gatcaaggcg agttacatga  
3421 tccccatgt tgtgcaaaaa agcggttagc tccttcggtc ctccgatcgt tgtcagaagt  
3481 aagttggccg cagtgttata actcatggtt atggcagcac tgcataattc tcttactgtc  
3541 atgcatccg taagatgctt ttctgtgact ggtgagtact caaccaagtc attctgagaa  
3601 tagtgtatgc ggcgaccgag ttgctcttgc ccggcgtcaa tacgggataa taccgcgcca  
3661 catagcagaa ctttaaaagt gctcatcatt ggaaaacgtt cttcggggcg aaaactctca  
3721 aggatcttac cgctggtgag atccagttcg atgtaacca ctctgtcacc caactgatct  
3781 tcagcatctt ttactttcac cagcgtttct gggtgagcaa aaacaggaag gcaaaatgcc  
3841 gcaaaaaagg gaataagggc gacacggaaa tgttgaatac tcatactctt ctttttcaa  
3901 tattattgaa gcatttatca gggttattgt ctcatgagcg gatacatatt tgaatgtatt  
3961 tagaaaaata aacaaatagg ggttccgcgc acatttccc gaaaagtgcc acctgacgtc  
4021 taagaaacca ttattatcat gacattaacc tataaaaata ggcgatcac gaggcccttt  
4081 cgtctcgcgc gtttcggtga tgacggtgaa aacctctgac acatgcagct cccggagacg  
4141 gtcacagctt gtctgtaagc ggatgccggg agcagacaag cccgtcaggg cgcgtcagcg  
4201 ggtggtggcg ggtgtcgggg ctggcttaac tatgcggcat cagagcagat tgtactgaga  
4261 gtgaccata tgcggtgta aataccgcac agatgcgtaa ggagaaaata ccgcatcagg  
4321 gcacattcgc cattcaggct gcgcaactgt tgggaagggc gatcgggtcg ggcctcttcg  
4381 ctattacgcc agctggcgaa aggggatgt gctgcaaggc gattaagttg ggtaacgcca  
4441 gggttttccc agtcacgacg ttgtaaaacg acggccagtg aattaattcg ttgcaggaca  
4501 ggatgtggtg cccgatgtga ctagctcttt gctgcaggcc gtcctatcct ctggttcga  
4561 taagagacc agaactccgg cccccaccg cccaccgcca ccccataca tatgtggtac  
4621 gcaagtaaga gtgcctgcgc atgccccatg tgccccacca agagctttgc atccataca  
4681 agtccccaaa gtggagaacc gaaccaattc ttcgcccggc gaacaaaagc ttctgcacac  
4741 gtctccactc gaatttggag ccggccggcg tgtgcaaaag aggtgaatcg aacgaaagac  
4801 ccgtgtgtaa agccgcggtt ccaaaatgta taaaaccgag agcatctggc caatgtgcat

4861 cagttgtggt cagcagcaaa atcaagtga tcatctcagt gcaactaaag ggggatcta  
4921 gatcggggta ccgccaccat gggtaagcct atccctaacc ctctcctcgg tctcgattct  
4981 acgactagtg gaggaggagg ttctggtggt ggtGCGCTTC CAGGCAACGG CATCTACGTG  
5041 GTGCGGGGCG AAATGGCCAC CCTGATGACG GCGATGCGAC GTGGAACGCG TTGGAATGCC  
5101 ACCGCCTACG TGGACGACGA GAATGACTCG CTGCTGAAGC TGTTATTGA CCTCAAGCAT  
5161 GAGCTAAATC GGATCGAGGA CCTGCGTCAG ATCGAGCCTC AGGTCTTCCT GGCTCCGTTT  
5221 CTTGAGGTGA TTCGCACGGC GGACGCCACG GGTCCGTTGA CTAGTCTAGC CTTGGCCTCG  
5281 GTTAACAAAT TATTGTCTTA CGGGCTAATA GATCCACGT CTCAAATCT GGCTGACATT  
5341 GTGGAGCGCA TTGCCGATGC TGTGACACAT GCCCGCTTTA TGGGCACCGA TCAGTCCCTCG  
5401 GATGGTGTCA CCTTTATGCG AGTGATTGAG GTGCTGCACA CGCTTATCCG CAGTCCCGAA  
5461 GGAGCCGCCG TCAGCAATGT GTCCATGTGC GAGGTGATGC TCAGTGCTT CAAGATCTCC  
5521 TTCGAGCCGA GGCTGAGTGA ACTGCTTCGT CGCTCGGCGG AAAAATCGCT CAAGGACATG  
5581 GTGCTGCTCT TCTTCATGCG CCTTCCCCAG TTCGCCGAAG AGCGAAGTGA CACCATGCTC  
5641 CAGAAGCGAT TTAATATCGG CGATGCTGCC AGCGGAGCTA CCCAAGAAAA ACTAAAGCGT  
5701 AAGACGGTTG CCCAAGCCCA GACAGCACCC AGGAAATCGT CAGCAGTGA GGAACCGCCT  
5761 CAAACACCGC AGTCTGCCAA CCTGACGGTG CCAGGGCACT TGAAGGCACC CATATTGGCC  
5821 ACCACACCCG CCAGTCCAGC GGGAAACATA TTGGACATGC AGGGCAAGAT TACGCAGACA  
5881 CCAACAACAA CGGCGAGCAC GGGGGAAGAT GAAACCACTG TCCCGGAGAC TCCTGTTATT  
5941 CAAGTGGAGT CGACTGAATC GGAGCCCTTG CTGGACGGCG AAACGGGTGA AGCAACCAGC  
6001 ACCCTGGCCG AGGCAAATAG TAGCGAGTAC ATCAACTCGG TGGGCGTCCG TTTTACACAG  
6061 CAGTCCACCG ATCACGATGT AACATCACTA TCCCCTTATG GCCTGCCCTT CATCCAGGAG  
6121 TTGTTCCGAT TCCTCATAAT CCTCTGCAAT CCTCTGGATA AGCAGAACTC GGATAGCATG  
6181 ATGCACACGG GTCTTAGTCT ACTTACTGTA GCTTTTGAAG TCGCAGCCGA TAATATTGGA  
6241 AAGTATGAGG GCTTGCTGGA GCTGGTTAAG GACGACTTAT GCAGAACTT AATATCGCTT  
6301 CTCAGCTCAG AGCGGCTTAG CATCTTTGCC GCCGATTTGC AGCTCTGTTT TTTGCTTTTC  
6361 GAGTCTCTCC GCGGACATCT CAAGTTTCAG CTGGAAGCCT ACCTTAGAAA ATTGAGCGAG  
6421 ATTATTGCTA GCGATAATCC CAAGACGCC TACGAAATGC GAGAACTCGC TCTGGACAAT  
6481 CTAAGTGCAGT TGTGGCGCAT TCCCGGCTTC GTCACGGAAT TGTATATCAA CTACGATTGT  
6541 GACTTGACT GCACGGATAT GTTTGAAAGT TTGACAAACC TACTGAGCAA GTATACGCTG  
6601 TCAGCAACGA ATGAGTTTA TAGCACCCAC ATTATCTCAA TGGACACCCT GTTAAGTGTG  
6661 ATAGACAGTA TCGAGCGAAA TTGTGCCGCG AGCAAGAATA GCAGCAACAA CAGAGAGTCC  
6721 TTGCCAGAAG CTGCCCCAGC AACAGGTGGC AGCCGCCATT CTCGCCACAA CAGCGGATTG  
6781 GAGGAATCG TAATTGATTC TGGCAATAGT GTAGCTGCAG AAGAGAAAGT GGAGAATC  
6841 GCAAGCTTTA TAAATGCGAG CTCACATCGA CTACGACTAC AATCTGGCGG AGAGGGAGTG  
6901 GGAATAACCA GTGAACAGCT GGCCAAGGTC AAACAGAAGA AGCGTCTGCT ATCCCAAGGC  
6961 ACAGAGCGAT TTAATCAGCG TCCAGAGAAA GGAATCCAGT ATCTGCAAGA ACACGGCATC

7021 CTAATGCCG AGCTTGATCC CATGCAGGTG GCCCTGTTCC TTCGAGAAAA TCCCGGGCTC  
7081 GATAAAAAA TGATTGGCGA ATATATCTCG AAAAAGAAAA ACGTCGACTC TAAGATTCTA  
7141 ATTAATTTTG TGGACTCGTT TGATTTCACT GGTCTTCGAG TGGATCAAGC ATTGCGTCTT  
7201 TATCTGGAGA CCTTCAGATT GCCCGGAaAG GCTCCATTGA TCTTTTTGGT GCTGGAACAC  
7261 TTTTCTGATC ATTGGCATAA ACAAACCAA GATCCGTTTG CCAACGTAGA CGCTGCTTTT  
7321 CGCTTGGCCT ATGCCATCAT CATGCTGAAC ATGGATCAGC ACAACTCGAA CGCGAAGCGT  
7381 TTAAATGTTT CAATGACGCT CGAGGACTTC ACTAAGAATT TGCCTGGTCT AAACGGTGGC  
7441 GAAGATTTTC ATCAAGAAAT GCTGGCTCAA GTCTTTAATG CAATCAAGAA CGAAGAGATC  
7501 GTTATGCCAG CAGAGCAAAC GGGTCTGGTG CGTGAAAATT ATCAATGGAA AGTACTGCTT  
7561 CGACGAGGAG ACACGCACGA TGGACATTTT CACTATGTGC ATGACGCATC ATACGACGTG  
7621 GAGATCTTCA ATATTGTGTG GGGTGCTTCT CTGAGCGCCC TAAGCTTTAT GTTTGATAAA  
7681 AGCACTGAAA CGGGCTACCA AAGAACTCTA GCAGGTTTCA GCAAATCCGC TGCCATATCG  
7741 GCGCACTATA ATCTGCATTC GGACTTCGAT GCCCTCGTTT TAACTCTCTG CAAATTCACA  
7801 ACGCTGCTGA GCAGCGTAGA ACAGCATGAG CCCGCTCCGG CGAACAATGA AACCCAGCAA  
7861 GCTGTGAACT TTGGATTGAA CGGAAAGGCT CAGGCTGCCA TGCGAACGGT GTTTCTATTG  
7921 GTTCACGACT ACGGCGATTG CTTAAGAGAG AGCTGGAAAC ACATTTTGGG CCTATATCTG  
7981 CAGCTTTTCC GTCTAAAGTT GCTGCCAAAA TCATTGATCG AAGTGGGAAGA CTTTTGTGAG  
8041 GCGAACGGAA AGGCCATGTT AATCCTGGAA AAGCCCCGCG AGAAGCAGGA ATCGGGACTA  
8101 TTTTCCAGCC TGTAATCATT TATCAGCTCG GAGGGTCAGC GAGAACCAAC GTACGAGGAG  
8161 CAGGACTTCA TCAAATGGG ACGGAAGTGC ATTAAGGAGT GCCAGCTGGA TCAAATGCTG  
8221 CAGGAATCAA AGTTTGTGCA ACTAGAGTCG CTGCAGGAGT TGCTTAAATG CGTTCTAGCG  
8281 CTAAGTGAAG CTCCTCAGGG GCACAAATCC ATTGGCCTGC CGTACGCCGA AGATCAAATC  
8341 GTTTTCTGGA TGGAATTTT GGTCAAGATA GTTGTTTATA ACCGGGATCG CATGATACCG  
8401 CTGTGGCCAG CAGTTCGAGA CCAAATGTAC CTAAGTCTTA TGGGCAGTGC CTCCTGTGGA  
8461 TACGACTACC TACTCAACCG ATGCATTGTA GCGGTCTTAA AACTAGCTAT CTATCTGATG  
8521 CGAAACGAAG AACTGTGTCC GATCGTATTG CAATCGCTCA AGATGCTTTT AATGCTTAAAG  
8581 CCAGCCTTGT TGCTGCGCAT TTCTAAACAG ATTTCCATTG GTATCTATGA GCTGCTCAAG  
8641 ACGTCGGCCC AAAATATTCA TTCCGAGCAG GACTGGCAGA TTATTTTCAA TCTACTTGAA  
8701 TGCGTGGGAG CCGGTGCTGT GCCGCCAAT TATGATGATG CCCAGCTGCC ATTGCCGCC  
8761 AACGGAAGTG CAAAGTCTGA TGGCGCTATA AGTGGCGAAG AGGACGCAAC TGCCGTGCCA  
8821 GAGCGTGGTT ACACTTCGGA TTCGGAGATC ACGAAAGCAT CTGCAGCACC TGCAGTCTCC  
8881 AGTCCAAGTG CTGAGAAGTG GATTCTGGTC AATAACAAGG ACAGTGAATT GACTACGGCC  
8941 TCTAGACCAC AATCTCCGCC TAGCTGAGT GCTCCTCCAG TAAATACGCT TGTGTACAAT  
9001 TGCCAGCTAC TAGACCACGC TCCGTTTGCT CTTTTCAAGT GCTGGGATTC GCTGGCGTTT  
9061 ATCGTGCGCA GTGTGGCACA CATCACGCCT TACAATTTTG AAGCCTGCGT TCGCTGCATC  
9121 CGCATCTTTG TGGAGGCTTG TCGGGATGGA GGTATACGCC AGCGGCGAAA GCTGGAATCG

9181	GCGGCTAAGC	AGAAAAGTTC	CAAGAAGCGC	AGCGAACGCA	AACCGGGCAT	GGCTTCCTCC
9241	GCCTCGAGTA	GTAATCTTAC	TCTTCTGACG	GGCGACCCGT	CCGACAACCA	GATAAACGGA
9301	AATGCGGCAG	AGCAGGAGGA	CCTGGCCCAG	CGCTACGAAC	AGTTGTCCAT	TCAACTGCTG
9361	GACCTGATGT	ATACGTTGTA	CACGCGAACT	GCCCAAATCT	TCCGATGGTG	GGCGGAAGAA
9421	GGATGCACAG	TGCCGCAGTC	GGCAGCTTTG	TGGTCACCGG	GCTGGTGTCC	ATTGCTTCAG
9481	GGAATCGCCA	GGCTGGCAAT	GGATCGACGG	CGAGAGGTGC	GCACCCATGC	CATATCGTGC
9541	CTGCAGCAGC	GGGCATTGCT	AGTCCATGAC	CTGCAAACGT	TGTCGGGAAC	GGAGTGGTGC
9601	TCTTGCTTCC	ACCAGGTGCT	GTTCCCCCTC	CTAAACGAAC	TGCTGCCCCG	GAGTAATGCA
9661	GCCGGCCAAC	TGGATGCCGC	TCTCCTCGAA	GAGTCGCGTA	TACGAACGGC	CACCATTATG
9721	TCTAAGGTGT	TCCTGCAACA	CCTGACCACG	CTCATCGAGC	TGGGAAATGC	TTTTAACGAG
9781	CTGTGGCTGG	ATATATTGGA	CTACATTGAG	CGCTTTATGA	AGGTGGGATC	GGACACATTG
9841	TCCGAGCAGA	TGCAGGAGAT	ACTGAAGAAC	ATGCTGCTGG	TGATGCATTC	AGTGCAGATG
9901	TTCCACAATC	AGGATGGTAG	TTTACAGCAG	GCTCTTTGGG	AGCTAACCTG	GCGACGCATC
9961	GCCGAATTTT	TGCCCAACCT	GAAGGAGGAG	CTTTTCCACG	ACGAAGGCAA	GCGAGCTCAG
10021	ACCTTAACGA	ACCCAGCTCC	ACAGGCAGCT	GTGGCTGCCG	CTCCACAGCA	ACAGTTACCA
10081	GCGGTGACCA	TTTTGCCCAG	GCAAACCCAG	GTTTCCAACG	AGTTAGTGGT	GAGCGCGCCT
10141	ACTCCGCCGG	CAGCCACACC	TTTGCTGGGC	TCTCCCGTCG	AGTCGCCGAG	GCGGAGCATA
10201	ATACTGCAGC	CACCCATGGC	CGATGTACTG	CAACAGCCGC	CCAGCTTGTG	ATTTGCTCAG
10261	CCCATTATTG	TGCCACCCCA	GCCGCCTGCA	GTTACGGACC	CAATACCACC	AAGTACATTA
10321	TTGCCGGATT	TGGTGAATGA	GGCAACTGCT	GCTGCCGTGC	AAGCCACGAC	CACGTCCCCG
10381	ACGCACAGCC	CGCAGGAGGC	GGAGCAGCCG	GCTTCAATAG	TGCAGCAGAC	CAACATCGTA
10441	ACCACCAACA	ATACGTACAA	TAGCTACGCC	ATTGAGGTGC	CCATGGCGCC	GGAGACAACT
10501	GCGGAACAGT	TTGGGCAGCA	GCAGCAGCAA	CTGCTTTACC	AACAGTACTA	TCAACAGTAT
10561	CAGGCCCAGC	AGCAACAGTT	GCCGGCTCCA	GCCAGCGACC	CTGCCATCAA	TGTGCCAATT
10621	AGTCATCTGC	TGGCCGAAA	TGCGTACCCC	TCGCTCCCCA	AAATGCCGCA	GGCATCCATT
10681	GTGCACAGCT	TTGCGCCCGT	TTACGAAAGC	CAGGCGGCGA	CGAGTGGAGC	TGGGACAGCA
10741	GCAGCGGACA	TCTATCAGGA	GTATGTGCAA	AATCCGTACA	ACCTTACGTT	GCAACAGCAT
10801	CCCCAACAGC	AGCTCCATCA	GCAGCAGCAG	CAGCAACAAC	AGCAGGCTAC	AGGAATGGCC
10861	AACGCATTTT	CCGCCGTTGC	CAGCCAGCC	AACTACTTTA	ATGTGAATGT	GGACCCCAGT
10921	AGCATACCGC	CCGATCGGA	ACTGCTCTAC	GGCCAGCAGT	AAcgcgtacc	ggtcatcatc
10981	accatcacca	ttgagtttaa	acccgctgat	cagcctcgac	tgtgccttct	aagatccaga
11041	catgataaga	tacattgatg	agtttggaca	aaccacaact	agaatgcagt	gaaaaaaaaatg
11101	ctttatttgt	gaaatttgtg	atgctattgc	tttatttcta	accatt	
//						
pMT-hyg-V5::iRFP713::myc						
LOCUS	MT-hyg-V5::iRFP713::myc	6293 bp	ds-DNA	circular	28-	

```
FEB-2020
DEFINITION pMT-puro Sequencing Result
  ORGANISM other sequences; artificial sequences; vectors.
COMMENT pMT-mAX2m-blast from 1 to 5500
COMMENT pMT-mAX2m-neo from 1 to 5813
COMMENT pMT-mAPEX2m from 1 to 5561
COMMENT pMT-mSOG1m from 1 to 5078
COMMENT pMT-mSOG4m from 1 to 5114
COMMENT ApEinfo:methylated:1
FEATURES             Location/Qualifiers
  misc_feature       complement(6204..6223)
                    /label=EBV_rev_primer
                    /ApEinfo_fwdcolor=#ff3600
                    /ApEinfo_revcolor=#ff3600
                    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                    width 5 offset 0
  CDS                5035..5982
                    /codon_start=1
                    /product="phytochrome-based near-infrared fluorescent
                    protein, originally termed iRFP (Filonov et al., 2011)"
                    /note="name was changed to iRFP713 (Shcherbakova and
                    Verkhusha, 2013)"

/translation="MAEGSVARQPDLLTCDDEPIHIPGAIQPHGLLLALAADMTIVAG
              S
DNLPELTGLAIGALIGRSAADVDFSETHNRLTIALAEPGAAVGAPITVGFTMRKDAGF
              I
GSWHRHDQLIFLELEPPQRDVAEPQAFFRRTNSAIRRLQAAETLESACAAAAQEVRKI
              T
GFDRVMIIYRFASDFSGEVIAEDRCAEVESKGLGHYPASTVPAQARRLYTINPVRIIPD
              I
NYRPVPVTPDLNPVTGRPIDLFAILRSVSPVHLEFMRNIGMGTMSISILRGERLWG
```

```
L IVCHHRTPYYVDLDGRQACELVAQVLAWQIGVMEE"  
/label=iRFP713  
/ApEinfo_fwdcolor=#9a69fe  
/ApEinfo_revcolor=#cde7f7  
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}  
width 5 offset 0  
regulatory    5029..5038  
/regulatory_class="other"  
/note="vertebrate consensus sequence for strong  
initiation  
of translation (Kozak, 1987)"  
/label=vertebrate consensus sequence for strong  
initiation  
of translation (Kozak, 1987)  
/ApEinfo_fwdcolor=pink  
/ApEinfo_revcolor=pink  
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}  
width 5 offset 0  
misc_feature  complement(6161..6178)  
/label=BGH_rev_primer  
/ApEinfo_fwdcolor=#ff3600  
/ApEinfo_revcolor=#ff3600  
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}  
width 5 offset 0  
misc_feature  4942..4983  
/label=V5  
/ApEinfo_fwdcolor=#ffffcc  
/ApEinfo_revcolor=#ffffcc  
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}  
width 5 offset 0  
misc_feature  6121..6138  
/label=6xHis  
/ApEinfo_fwdcolor=cyan  
/ApEinfo_revcolor=#00ff00  
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}  
width 5 offset 0
```



```
protein_bind    6025..6054
                /label=Myc Tag EQKLISEEDL
                /ApEinfo_fwdcolor=#ffcc66
                /ApEinfo_revcolor=#ffcc66
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
misc_feature    4990..5013
                /label=Linker GL3
                /ApEinfo_fwdcolor=#ffcc66
                /ApEinfo_revcolor=#ffcc66
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
misc_feature    6018..6023
                /label=XhoI
                /ApEinfo_fwdcolor=#ffffcc
                /ApEinfo_revcolor=#ffffcc
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
misc_feature    5992..6015
                /label=Linker GL4
                /ApEinfo_fwdcolor=#ffcc66
                /ApEinfo_revcolor=#ffcc66
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
misc_feature    4984..4989
                /label=SpeI
                /ApEinfo_fwdcolor=#ffffcc
                /ApEinfo_revcolor=#ffffcc
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
misc_feature    complement(225..1250)
                /label=Hyg
                /ApEinfo_fwdcolor=#ffffcc
                /ApEinfo_revcolor=#00ff00
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
```

```
gene      complement(143..173)
          /gene="puro (variant)"

/translation="MGSCAPFGRALRVVGRASGTGLAGHAPGARSFGHLDVGGDGEAE
PLVEGEVAGRGGLQEGGHPGALGRLHSGEHDGAAQTLALVVGRDADGGQEPRGLLGPV
RRQEAFHLLLRGQPFTAQLGHARADLGEHRPRFDALRRGPDRHRGAVVRDPHLADVEP
DAREEEFLQLGDPLDVAVRVDGVARGGVVGERGGEGAYGPGDVVAGGEAHRGLVLGHG
          RSPCEGSGAWVRGWRRHSWRPTCRHASYRIPAARGICCNL*"
          /label=puro (variant)
          /ApEinfo_fwdcolor=#ffffcc
          /ApEinfo_revcolor=#ffffcc
          /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
          width 5 offset 0
misc_feature  95..115
          /label=New Feature
          /ApEinfo_fwdcolor=cyan
          /ApEinfo_revcolor=#00ff00
          /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
          width 5 offset 0
misc_feature  4892..4909
          /label=Metallothionein_primer
          /ApEinfo_fwdcolor=#ff3600
          /ApEinfo_revcolor=#ff3600
          /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
          width 5 offset 0
promoter     4463..4479
          /label=M13_forward20_primer
          /ApEinfo_fwdcolor=#ccffed
          /ApEinfo_revcolor=#ccffed
          /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
          width 5 offset 0
misc_feature  4448..4470
          /label=M13_pUC_fwd_primer
```

```

    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
promoter    complement(3926..3954)
    /label=AmpR_promoter
    /ApEinfo_fwdcolor=#ccffed
    /ApEinfo_revcolor=#ccffed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
rep_origin  complement(2250..2869)

/translation="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGY
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHVTRL
DRWEPELNEAIPNDERDTMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPL
LRSALPAGWFIADKSGAGERSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
    EIGASLIKHW*"
    /label=pBR322_origin
    /ApEinfo_fwdcolor=pink
    /ApEinfo_revcolor=pink
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature complement(1876..1898)

/translation="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGY
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHVTRL
DRWEPELNEAIPNDERDTMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPL
```

```
LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
    EIGASLIKHW*"
    /label=M13_pUC_rev_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    complement(1350..1708)
    /label=Copia Promoter?
    /ApEinfo_fwdcolor=#cde7f7
    /ApEinfo_revcolor=#cde7f7
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    complement(4327..4482)
    /label=lacZ_a
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    complement(4113..4135)
    /label=pGEX_3_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
gene            complement(3024..3884)
    /gene="Ampicillin"
    /label=Ampicillin
    /ApEinfo_fwdcolor=#ffffcc
    /ApEinfo_revcolor=#ffffcc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
promoter        complement(1912..1941)

/translation="MSIQHFRVALIPFFAAFLP VF AHPETLVKVKDAEDQLGARVGY
```

```
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLTTIGGPKELTAFLHNMGDHSVTRL
DRWEPELNEAIPNDERDTMPVAMATTLRKLTTGELLTLASRQQLIDWMEADKVAGPL
LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
    EIGASLIKHW*"
    /label=lac_promoter
    /ApEinfo_fwdcolor=#ccffed
    /ApEinfo_revcolor=#ccffed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
promoter      complement(1859..1877)

/translation="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGY
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLTTIGGPKELTAFLHNMGDHSVTRL
DRWEPELNEAIPNDERDTMPVAMATTLRKLTTGELLTLASRQQLIDWMEADKVAGPL
LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
    EIGASLIKHW*"
    /label=M13_reverse_primer
    /ApEinfo_fwdcolor=#ccffed
    /ApEinfo_revcolor=#ccffed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature  4489..4856
    /label=MT-promoter
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
```

```
width 5 offset 0
CDS      complement(4323..4391)
        /label=LacZ alpha
        /ApEinfo_fwdcolor=#6495ed
        /ApEinfo_revcolor=#6495ed
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind 3998..4015
        /label=pQE60-F
        /ApEinfo_fwdcolor=#ff00bd
        /ApEinfo_revcolor=#ff0003
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind  complement(1859..1879)
        /label=M13R_GW
        /ApEinfo_fwdcolor=#ff00bd
        /ApEinfo_revcolor=#ff0003
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind  complement(1859..1879)
        /label=M13-rev
        /ApEinfo_fwdcolor=cyan
        /ApEinfo_revcolor=green
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind  4462..4479
        /label=M13-fwd
        /ApEinfo_fwdcolor=cyan
        /ApEinfo_revcolor=green
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind  4436..4459
        /label=M13F
        /ApEinfo_fwdcolor=#0a00ff
        /ApEinfo_revcolor=#0a00ff
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
```

```
width 5 offset 0
misc_feature 4927..4932
  /label=KpnI
  /ApEinfo_fwdcolor=#ffffcc
  /ApEinfo_revcolor=#ffffcc
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind 4832..4849
  /label=SEQ-MT-F2
  /ApEinfo_fwdcolor=#ff00bd
  /ApEinfo_revcolor=#ff0003
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
rep_origin complement(2247..2929)
  /label=ColE1 origin
  /ApEinfo_fwdcolor=gray50
  /ApEinfo_revcolor=gray50
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
CDS complement(3027..3686)
  /label=AmpR
  /ApEinfo_fwdcolor=yellow
  /ApEinfo_revcolor=yellow
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_binding complement(1885..1907)
  /label=Lac0
  /ApEinfo_fwdcolor=#6495ed
  /ApEinfo_revcolor=#6495ed
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind complement(3605..3629)
  /label=Amp-GF
  /ApEinfo_fwdcolor=#ff00bd
  /ApEinfo_revcolor=#ff0003
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
```

```
width 5 offset 0
primer_bind    3147..3170
               /label=Amp-GR1
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    4462..4479
               /label=M13F_GW
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    1737..1759
               /label=Seq-EB-R
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature   1254..1268
               /label=New Feature(1)
               /ApEinfo_label=New Feature
               /ApEinfo_fwdcolor=cyan
               /ApEinfo_revcolor=#00ff00
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
```

ORIGIN

```
1 ataagctgca ataaacaagt tctagagtcg gtgggcctcg ggggcgggtg cggggtcggc
61 ggggccgcc cgggtggctt cggtcggagc catggggtcg tgcgctcctt tcggtcgggc
121 gctgcgggtc gtggggcggg cgtcaggcac cgggcttgcg ggtcatgcac cagttgctgg
181 ctgaggcggt ctcgaaatca gctcttgttc ggtcggcatc tactctattc ctttgccctc
241 ggacgagtg cggggcgctc gtttccacta tcggcgagta cttctacaca gccatcggtc
301 cagacggccg cgcttctgcy ggcgatttgt gtacgcccga cagtcccggc tccggatcgg
361 acgattgcgt cgcacgacc ctgcgccaa gctgcatcat cgaaattgcc gtcaaccaag
421 ctctgataga gttggtcaag accaatgcgg agcatatacg cccggagccg cggcgatcct
481 gcaagctccg gatgcctccg ctcgaagtag cgcgtctgct gctccataca agccaaccac
```



541 ggctccaga agaagatgtt ggcgacctg tattgggaat ccccgaacat cgctcgctc  
601 cagtcaatga ccgctgttat gcggccattg tccgtcagga cattgttggg gccgaaatcc  
661 gcgtgcacga ggtgccggac ttcggggcag tcctcggccc aaagcatcag ctcatcgaga  
721 gcctgcgcga cggacgcact gacggtgtcg tccatcacag tttgccagtg atacacatgg  
781 ggatcagcaa tcgcgcatat gaaatcacgc catgtagtgt attgaccgat tccttgcggt  
841 ccgaatgggc cgaacccgct cgtctggcta agatcggccg cagcgatcgc atccatggcc  
901 tccgcgaccg gctgcagaac agcgggcagt tcggtttcag gcaggctctg caacgtgaca  
961 ccctgtgcac ggcgggagat gcaataggtc aggtctctgc tgaattcccc aatgtcaagc  
1021 acttccggaa tcgggagcgc ggccgatgca aagtgccgat aaacataacg atctttgtag  
1081 aaaccatcgg cgcagctatt taccgcagg acatatccac gccctcctac atcgaagctg  
1141 aaagcacgag attcttcgcc ctccgagagc tgcacaggt cggagacgct gtcgaacttt  
1201 tcgatcagaa acttctcgac agacgtcgcg gtgagttcag gctttttcat ggtggtcgtc  
1261 tccttgtgag gggtcagggg cgtgggtcag gggatggtgg cggcaccggt cgtggcggcc  
1321 gacctgcagg catgcaagct atcgaattcc tgcagcccgg gggatctgtt gtaatttata  
1381 atttatattt ctttcttaa taaataaata aatagtcaag tttatgtttg agttttatga  
1441 tttatatttt aagttatttc aactgcaaca ccagcaccac gacctactta cagcaaaaaa  
1501 cgtacaagaa ggaagaagg aataaaaaga gtggtattct cttacaatat gttttatggc  
1561 ataaaagggt tggccattca tatcaaatat aaagtagtgt tgtttaacgt tacttttcta  
1621 ggttgaatag tatattccaa cagatgatga ggggttccca atcctaacc catttgccgt  
1681 tcccagaagc atgaaaccac cacgcaccgg atcctctaga acaacaacaa ttgcattcat  
1741 tttatgtttc aggttcaggg ggaggtgtgg gaggtttttt aaagcaagta aaacctctac  
1801 aatgttggtg tggctgatta tgatcagtcg acctgcaggc atgcaagctt ggcgtaatca  
1861 tggcatagc tgtttcctgt gtgaaattgt tatccgctca caattccaca caacatacga  
1921 gccggaagca taaagtgtaa agcctggggg gcctaagtag tgagctaact cacattaatt  
1981 gcgttgcgct cactgcccgc tttccagtcg ggaacctgt cgtgccagct gcattaatga  
2041 atcggccaac gcgcggggag aggcggtttg cgtattgggc gctcttcgc ttctcgctc  
2101 actgactcgc tgcgctcggc cgttcggctg cggcgagcgg tatcagctca ctcaaaggcg  
2161 gtaatacggc tatccacaga atcaggggat aacgcaggaa agaacatgtg agcaaaaggc  
2221 cagcaaaagg ccaggaaccg taaaaggcc gcgttgctgg cgtttttcca taggctccgc  
2281 cccctgacg agcatcaca aatcgacgc tcaagtcaga ggtggcgaaa cccgacagga  
2341 ctataaagat accaggcgtt tccccctgga agctccctcg tgcgctctcc tgttccgacc  
2401 ctgccgctta ccggatacct gtccgccttt ctcccttcgg gaagcgtggc gctttctcat  
2461 agctcacgct gtaggtatct cagttcgggt taggtcgttc gctccaagct gggctgtgtg  
2521 cacgaacccc ccgttcagcc cgaccgctgc gccttatccg gtaactatcg tcttgagtcc  
2581 aacccggtaa gacacgactt atcgccactg gcagcagcca ctggtaacag gattagcaga  
2641 gcgaggatg taggcggtgc tacagagttc ttgaagtggt ggcctaacta cggctacact

2701 agaagaacag tatttggtat ctgcgctctg ctgaagccag ttaccttcgg aaaaagagtt  
2761 ggtagctctt gatccggcaa acaaacacc gctggtagcg gtggtttttt tgtttgcaag  
2821 cagcagatta cgcgagaaa aaaaggatct caagaagatc ctttgatctt ttctacgggg  
2881 tctgacgctc agtggaacga aaactcacgt taagggattt tggatcatgag attatcaaaa  
2941 aggatcttca cctagatcct tttaaattaa aatgaagtt ttaaatcaat ctaaagtata  
3001 tatgagtaaa cttggtctga cagttaccaa tgcttaatca gtgaggcacc tatctcagcg  
3061 atctgtctat ttcgttcatc catagttgcc tgactccccg tcgtgtagat aactacgata  
3121 cgggagggct taccatctgg cccagtgct gcaatgatac cgcgagacc acgctcaccg  
3181 gctccagatt tatcagcaat aaaccagcca gccggaagg ccgagcgag aagtggctct  
3241 gcaactttat ccgctccat ccagtctatt aattggtgcc ggaagctag agtaagtagt  
3301 tcgccagtta atagtttgcg caacgttggt gccattgcta caggcatcgt ggtgtcacgc  
3361 tcgtcgtttg gtatggcttc attcagctcc ggttcccaac gatcaaggcg agttacatga  
3421 tccccatgt tgtgcaaaaa agcggttagc tccttcggtc ctccgatcgt tgtcagaagt  
3481 aagttggccg cagtgttatc actcatggtt atggcagcac tgcataattc tcttactgtc  
3541 atgcatccg taagatgctt ttctgtgact ggtgagtact caaccaagtc attctgagaa  
3601 tagtgtatgc ggcgaccgag ttgctcttgc ccggcgtcaa tacgggataa taccgcgcca  
3661 catagcagaa ctttaaaagt gctcatcatt ggaaaacgtt cttcggggcg aaaactctca  
3721 aggatcttac cgctggtgag atccagttcg atgtaacca ctctgtcacc caactgatct  
3781 tcagcatctt ttactttcac cagcgtttct gggtgagcaa aaacaggaag gcaaaatgcc  
3841 gcaaaaaagg gaataagggc gacacggaaa tgttgaatac tcatactctt ctttttcaa  
3901 tattattgaa gcatttatca gggttattgt ctcatgagcg gatataatt tgaatgtatt  
3961 tagaaaaata aacaaatagg ggttccgcgc acatttccc gaaaagtgcc acctgacgtc  
4021 taagaaacca ttattatcat gacattaacc tataaaaata ggcgtatcac gaggcccttt  
4081 cgtctcgcgc gtttcggtga tgacggtgaa aacctctgac acatgcagct cccggagacg  
4141 gtcacagctt gtctgtaagc ggatgccggg agcagacaag cccgtcaggg cgcgtcagcg  
4201 ggtggtggcg ggtgtcgggg ctggcttaac tatgcccagat cagagcagat tgtactgaga  
4261 gtgcaccata tgcggtgtga aataccgcac agatgcgtaa ggagaaaata ccgcatcagg  
4321 gcaccattcg cattcaggct gcgcaactgt tgggaagggc gatcgggtcg ggcctcttcg  
4381 ctattacgcc agctggcgaa aggggatgt gctgcaaggc gattaagttg ggtaacgcca  
4441 gggttttccc agtcacgacg ttgtaaaacg acggccagtg aattaattcg ttgcaggaca  
4501 ggatgtggtg cccgatgtga ctagctcttt gctgcaggcc gtcctatcct ctggttcga  
4561 taagagacc agaactccgg cccccaccg cccaccgcca ccccataca tatgtggtac  
4621 gcaagtaaga gtgcctgcgc atgccccatg tgccccacca agagctttgc atccataca  
4681 agtccccaaa gtggagaacc gaaccaattc ttcgcccgca gaacaaaagc ttctgcacac  
4741 gtctccactc gaatttgag ccggccggcg tgtgcaaaag aggtgaatcg aacgaaagac  
4801 ccgtgtgtaa agccgcgttt caaaatgta taaaaccgag agcatctggc caatgtgcat

```

4861 cagttgtggt cagcagcaaa atcaagtgaa tcatctcagt gcaactaaag ggggatcta
4921 gatcggggta ccgccaccAT GGGTAAGCCT ATCCCTAACC CTCTCCTCGG TCTCGATTCT
4981 ACGACTAGTg gaggaggagg ttctggtggt ggtGCGGCCG Catctgccgc caccATGGCT
5041 GAAGGATCCG TCGCCAGGCA GCCTGACCTC TTGACCTGCG ACGATGAGCC GATCCATATC
5101 CCCGGTGCCA TCCAACCGCA TGGACTGCTG CTCGCCCTCG CCGCCGACAT GACGATCGTT
5161 GCCGGCAGCG ACAACCTTCC CGAACTCACC GGACTGGCGA TCGGCGCCCT GATCGGCCGC
5221 TCTGCGGCCG ATGTCTTCGA CTCGGAGACG CACAACCGTC TGACGATCGC CTTGGCCGAG
5281 CCCGGGGCGG CCGTCGGAGC ACCGATCACT GTCGGTTC ACGATGCGAAA GGACGCAGGC
5341 TTCATCGGCT CCTGGCATCG CCATGATCAG CTCATCTTCC TCGAGCTCGA GCCTCCCCAG
5401 CGGGACGTCG CCGAGCCGCA GGCGTTCTTC CGCCGCACCA ACAGCGCCAT CCGCCGCCTG
5461 CAGGCCGCCG AAACCTTGA AAGCGCTGC GCCGCCGCGG CGCAAGAGGT GCGGAAGATT
5521 ACCGGCTTCG ATCGGGTGAT GATCTATCGC TTCGCCTCCG ACTTCAGCGG CGAAGTGATC
5581 GCAGAGGATC GGTGCGCCGA GGTCGAGTCA AAAGTAGGCC TGCACTATCC TGCCTCAACC
5641 GTGCCGGCGC AGGCCCGTCG GCTCTATACC ATCAACCCGG TACGGATCAT TCCCGATATC
5701 AATTATCGGC CGGTGCCGGT CACCCAGAC CTCAATCCGG TCACCGGGCG GCCGATTGAT
5761 CTTAGCTTCG CCATCCTGCG CAGCGTCTCG CCCGTCCATC TGGAATTCAT GCGCAACATA
5821 GGCATGCACG GCACGATGTC GATCTCGATT TTGCGCGGCG AGCGACTGTG GGGATTGATC
5881 GTTTGCCATC ACCGAACGCC GTACTACGTC GATCTCGATG GCCGCCAAGC CTGCGAGCTA
5941 GTCGCCCAGG TTCTGGCCTG GCAGATCGGC GTGATGGAAG AGGGCGGCC tGGTGGTGA
6001 GTTTCTGGTg gcggtGGCTC GAGTGAGCAA AAGCTCATT CTGAAGAGGA CTTGTAAGgg
6061 cccttcgaag gtaagcctat ccctaaccct ctctcggtc tcgattctAC Gcgtaccggt
6121 CATCATCACC ATCACCATTG Agtttaaacc cgctgatcag cctcgactgt gccttctaag
6181 atccagacat gataagatac attgatgagt ttggacaaac cacaactaga atgcagtgaa
6241 aaaaatgctt tatttgtgaa atttgtgatg ctattgcttt atttgtaacc att

```

//

**pMT-V5-i713-Sec71**

LOCUS	pMT-V5-i713-Sec71	10266 bp ds-DNA	circular	28-FEB-2020
DEFINITION	pMT-puro Sequencing Result			
ORGANISM	other sequences; artificial sequences; vectors.			
COMMENT	pMT-vAX2m from 1 to 5573			
COMMENT	pMT-vSOG4m from 1 to 5126			
COMMENT	pMT-mSOGm_v4 from 1 to 5114			
COMMENT	ApEinfo:methylated:1			
FEATURES	Location/Qualifiers			
	misc_feature	complement(10177..10196)		

```
    /label=EBV_rev_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature    complement(10134..10151)
    /label=BGH_rev_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature    10094..10111
    /label=6xHis
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature    10043..10084
    /label=V5
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
CDS
    5014..9969
    /label=Sec71
    /ApEinfo_fwdcolor=#99ccff
    /ApEinfo_revcolor=#cde7f7
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature    9567..9567
    /label=T>G silent
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature    10031..10036
```

```
    /label=ApaI GGGCC^C
    /ApEinfo_fwdcolor=#ffffcc
    /ApEinfo_revcolor=#ffffcc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    7770..7789
    /label=sec71-GF6
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature   7629..7629
    /label=A>G in 40A
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature   4927..4932
    /label=KpnI
    /ApEinfo_fwdcolor=#ffffcc
    /ApEinfo_revcolor=#ffffcc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
protein_bind   6733..7305
    /label=Sec7 domain
    /ApEinfo_fwdcolor=#0080ff
    /ApEinfo_revcolor=#0080ff
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature   9552..9552
    /label=C>T silent
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    7250..7273
```

```
    /label=Sec71-DRSC01893-F (8A10)
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    9054..9073
    /label=sec71-GF8
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    8509..8530
    /label=sec71-GF7
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    complement(8562..8583)
    /label=Sec71-GR3
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature    6069..6069
    /label=C>T confirmed in 40A 2013
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature    10011..10030
    /label=New Feature
    /ApEinfo_fwdcolor=#ffffcc
    /ApEinfo_revcolor=#ffffcc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature    7557..7557
```

```

    /label=T>C in 40A
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    7668..7668
    /label=A>C in 40A
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    9662..9679
    /label=Sec71-GF10
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    7197..7215
    /label=Sec71-GF11
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    6797..6820
    /label=Sec71-GF12
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    7159..7161
    /label=M717(M194 in sec7 domain M>L = BFA-resistant)
    /label=M717L(BFA-resistant)
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
```

```
primer_bind    6903..6925
               /label=Sec71-GF14
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
misc_feature   complement(7099..7121)
               /label=Sec71-crRNA7(Protospacer)
               /ApEinfo_fwdcolor=#ccff66
               /ApEinfo_revcolor=#ffff9f
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
misc_feature   7188..7207
               /label=Sec71-crRNA8 Protospacer
               /ApEinfo_fwdcolor=#ccff66
               /ApEinfo_revcolor=#ffffcc
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
misc_feature   7828..7828
               /label=T>C Val>Ala in FRT40A
               /ApEinfo_fwdcolor=#ccff66
               /ApEinfo_revcolor=#00ff00
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
CDS            7890..7890
               /label=Sec71-PA
               /ApEinfo_fwdcolor=#99ccff
               /ApEinfo_revcolor=#cde7f7
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
CDS            5977..5979
               /label=Sec71-PA(1)
               /ApEinfo_label=Sec71-PA
               /ApEinfo_fwdcolor=#99ccff
               /ApEinfo_revcolor=#cde7f7
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
```



```
width 5 offset 0
misc_feature 9363..9363
  /label=T>C silent
  /ApEinfo_fwdcolor=cyan
  /ApEinfo_revcolor=#00ff00
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 8509..8530
  /label=Chang-F1
  /ApEinfo_fwdcolor=cyan
  /ApEinfo_revcolor=#00ff00
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind 5568..5587
  /label=sec71-GF3
  /ApEinfo_fwdcolor=#ff00bd
  /ApEinfo_revcolor=#ff0003
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 7890..7890
  /label=A>C silent
  /ApEinfo_fwdcolor=cyan
  /ApEinfo_revcolor=#00ff00
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
CDS 6069..6069
  /label=Sec71-PA(2)
  /ApEinfo_label=Sec71-PA
  /ApEinfo_fwdcolor=#99ccff
  /ApEinfo_revcolor=#cde7f7
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
CDS 6600..6600
  /label=Sec71-PA(3)
  /ApEinfo_label=Sec71-PA
  /ApEinfo_fwdcolor=#99ccff
```

```

/ApEinfo_revcolor=#cde7f7
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 9447..9447
/label=G>A silent
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 7150..7152
/label=S191
/ApEinfo_fwdcolor=#66ff66
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 9751..9751
/label=T>C silent(1)
/ApEinfo_label=T>C silent
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind 6550..6569
/label=Sec71-GF5
/ApEinfo_fwdcolor=#ff00bd
/ApEinfo_revcolor=#ff0003
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind complement(7726..7761)
/label=Sec71-DRSC01893-R
/ApEinfo_fwdcolor=#ff00bd
/ApEinfo_revcolor=#ff0003
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 9324..9324
/label=T>C silent(2)
```

```
    /ApEinfo_label=T>C silent
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    complement(9224..9245)
    /label=Chang-R1
    /ApEinfo_fwdcolor=#fb53d0
    /ApEinfo_revcolor=#fc5a5d
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    5924..5943
    /label=sec71-GF4
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature    7039..7041
    /label=E677 (E740 in garz)
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature    6600..6600
    /label=G>A silent(1)
    /ApEinfo_label=G>A silent
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature    7147..7149
    /label=F190 F190Y=BFA-hypersensitive
    /label=F713Y(BFA-sensitive)
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
```

```
width 5 offset 0
misc_feature 7719..7719
  /label=A>G 40A
  /ApEinfo_fwdcolor=cyan
  /ApEinfo_revcolor=#00ff00
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature 9336..9336
  /label=T>C in 40A(1)
  /ApEinfo_label=T>C in 40A
  /ApEinfo_fwdcolor=cyan
  /ApEinfo_revcolor=#00ff00
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind 5040..5057
  /label=GF9
  /ApEinfo_fwdcolor=#ff00bd
  /ApEinfo_revcolor=#ff0003
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind complement(7375..7398)
  /label=Sec71-GR4
  /ApEinfo_fwdcolor=#ff00bd
  /ApEinfo_revcolor=#ff0003
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind 8137..8155
  /label=Sec71-GF13
  /ApEinfo_fwdcolor=#ff00bd
  /ApEinfo_revcolor=#ff0003
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature 9970..9972
  /label=STOP
  /ApEinfo_fwdcolor=#66ccff
  /ApEinfo_revcolor=#00ff00
```

```
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    7073..7094
    /label=Sec71-GF15
    /ApEinfo_fwdcolor=#fb53d0
    /ApEinfo_revcolor=#fc5a5d
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature   7099..7101
    /label=PAM
    /ApEinfo_fwdcolor=#fc81f0
    /ApEinfo_revcolor=#fc81f0
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature   7208..7210
    /label=PAM(1)
    /ApEinfo_label=PAM
    /ApEinfo_fwdcolor=#fc81f0
    /ApEinfo_revcolor=#fc81f0
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature   4942..4983
    /label=V5(1)
    /ApEinfo_label=V5
    /ApEinfo_fwdcolor=#ffffcc
    /ApEinfo_revcolor=#ffffcc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature   4984..4989
    /label=SpeI
    /ApEinfo_fwdcolor=#ffffcc
    /ApEinfo_revcolor=#ffffcc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature   4990..5013
    /label=Linker GL3
```

```
    /ApEinfo_fwdcolor=#ffcc66
    /ApEinfo_revcolor=#ffcc66
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    4892..4909
    /label=Metallothionein_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    complement(4327..4482)
    /label=lacZ_a
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
promoter        4463..4479
    /label=M13_forward20_primer
    /ApEinfo_fwdcolor=#ccffed
    /ApEinfo_revcolor=#ccffed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    4448..4470
    /label=M13_pUC_fwd_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    complement(4113..4135)
    /label=pGEX_3_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
promoter        complement(3926..3954)
    /label=AmpR_promoter
```

```

    /ApEinfo_fwdcolor=#ccffed
    /ApEinfo_revcolor=#ccffed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
gene      complement(3024..3884)
    /gene="Ampicillin"
    /label=Ampicillin
    /ApEinfo_fwdcolor=#ffffcc
    /ApEinfo_revcolor=#ffffcc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
rep_origin  complement(2250..2869)

/translation="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGY
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHVTRL
DRWEPELNEAIPNDERDTMPVAMATTLRKLTTGELLTLASRQQLIDWMEADKVAGPL
LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
    EIGASLIKHW*"
    /label=pBR322_origin
    /ApEinfo_fwdcolor=pink
    /ApEinfo_revcolor=pink
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
promoter   complement(1912..1941)

/translation="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGY
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHVTRL
```

```
DRWEPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPL

LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
    EIGASLIKHW*"
    /label=lac_promoter
    /ApEinfo_fwdcolor=#ccffed
    /ApEinfo_revcolor=#ccffed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    complement(1876..1898)

/translation="MSIQHFRVALIPFFAAFCPLVFAHPETLVKVKDAEDQLGARVGY

IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE

YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLTTIGGPKELTAFLHNMGDHVTRL

DRWEPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPL

LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
    EIGASLIKHW*"
    /label=M13_pUC_rev_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
promoter        complement(1859..1877)

/translation="MSIQHFRVALIPFFAAFCPLVFAHPETLVKVKDAEDQLGARVGY

IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE

YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLTTIGGPKELTAFLHNMGDHVTRL

DRWEPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPL
```



```
LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
    EIGASLIKHW*"
    /label=M13_reverse_primer
    /ApEinfo_fwdcolor=#ccffed
    /ApEinfo_revcolor=#ccffed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    4489..4856
    /label=MT-promoter
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    complement(1350..1708)
    /label=Copia Promoter?
    /ApEinfo_fwdcolor=#cde7f7
    /ApEinfo_revcolor=#cde7f7
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    4462..4479
    /label=M13-fwd
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=green
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    complement(1859..1879)
    /label=M13-rev
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=green
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
rep_origin    complement(2247..2929)
    /label=ColE1 origin
    /ApEinfo_fwdcolor=gray50
    /ApEinfo_revcolor=gray50
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
```

```
width 5 offset 0
CDS      complement(4323..4391)
        /label=LacZ alpha
        /ApEinfo_fwdcolor=#6495ed
        /ApEinfo_revcolor=#6495ed
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_binding  complement(1885..1907)
        /label=Lac0
        /ApEinfo_fwdcolor=#6495ed
        /ApEinfo_revcolor=#6495ed
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
CDS      complement(3027..3686)
        /label=AmpR
        /ApEinfo_fwdcolor=yellow
        /ApEinfo_revcolor=yellow
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind  4436..4459
        /label=M13F
        /ApEinfo_fwdcolor=#0a00ff
        /ApEinfo_revcolor=#0a00ff
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind  4462..4479
        /label=M13F_GW
        /ApEinfo_fwdcolor=#ff00bd
        /ApEinfo_revcolor=#ff0003
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind  complement(1859..1879)
        /label=M13R_GW
        /ApEinfo_fwdcolor=#ff00bd
        /ApEinfo_revcolor=#ff0003
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
```

```
width 5 offset 0
primer_bind    3998..4015
               /label=pQE60-F
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    complement(3605..3629)
               /label=Amp-GF
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    3147..3170
               /label=Amp-GR1
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature   complement(225..1250)
               /label=Hyg
               /ApEinfo_fwdcolor=#ffffcc
               /ApEinfo_revcolor=#00ff00
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature   1254..1268
               /label=New Feature(1)
               /ApEinfo_label=New Feature
               /ApEinfo_fwdcolor=cyan
               /ApEinfo_revcolor=#00ff00
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
gene           complement(143..173)
               /gene="puro (variant)"

/translation="MGSCAPFGRALRVVGRASGTGLAGHAPGARSFGHLDVGGDGAE
```

PLVEGEVAGRGGLEGGHPGALGRLHSGEHDGAAQTLALVVGRRDADGGQEPRGLLGPV

RRQEAFHLLLRGQPFTAQLGHARADLGEHRPRFDALRRGPDRHRGAVVRDPHLADVEP

DAREEEFLQLGDPLDVAVRVDGVARGGVVGERGGEGAYGPGDVVAGGEAHRGLVLGHG

RSSPCEGSGAWVRGWRRHSWRPTCRHASYPARAARGICCNL\*"

/label=puro (variant)

/ApEinfo\_fwdcolor=#ffffcc

/ApEinfo\_revcolor=#ffffcc

/ApEinfo\_graphicformat=arrow\_data {{0 1 2 0 0 -1}} {{}} 0}

width 5 offset 0

misc\_feature 95..115

/label=New Feature(2)

/ApEinfo\_label=New Feature

/ApEinfo\_fwdcolor=cyan

/ApEinfo\_revcolor=#00ff00

/ApEinfo\_graphicformat=arrow\_data {{0 1 2 0 0 -1}} {{}} 0}

width 5 offset 0

ORIGIN

1 ataagctgca ataaacaagt tctagagtcg gtgggcctcg ggggcgggtg cggggtcggc  
61 ggggccgcc cgggtggctt cggtcggagc catggggtcg tgcgctcctt tcggtcgggc  
121 gctgcggtc gtggggcggg cgtcaggcac cgggcttgcg ggtcatgcac cagttgctgg  
181 ctgaggcgtt ctgaaatca gctcttgctt ggtcggcatc tactctattc ctttgcctc  
241 ggacgagtc tggggcgtc gtttccacta tcggcgagta cttctacaca gccatcggtc  
301 cagacggccg cgcttctgcg ggcgattgt gtacgccca cagtcccggc tccggatcgg  
361 acgattgcgt cgcacgacc ctgcgccca gctgcatcat cgaaattgcc gtcaaccaag  
421 ctctgataga gttggtcaag accaatgcgg agcatatacg cccggagccg cggcgtcct  
481 gcaagctccg gatgcctccg ctggaagtag cgcgtctgct gctccataca agccaaccac  
541 ggcctccaga agaagatggt ggcgacctc tattgggaat ccccgaacat cgcctcgtc  
601 cagtcaatga ccgctgttat gcggccattg tccgtcagga cattgttggg gccgaaatcc  
661 gcgtgcacga ggtgccggac ttcggggcag tcctcggccc aaagcatcag ctcatcgaga  
721 gcctgcgcga cggacgact gacggtgtcg tccatcacag tttgccagtg atacacatgg  
781 ggatcagcaa tcgcgcatat gaaatcacgc catgtagtgt attgaccgat tccttgcggt  
841 ccgaatgggc cgaacccgct cgtctggcta agatcggccg cagcgatcgc atccatggcc  
901 tccgcgaccg gctgcagaac agcgggcagt tcggtttcag gcaggctctg caacgtgaca

```
961 ccctgtgcac ggcgggagat gcaataggtc aggctctcgc tgaattcccc aatgtcaagc
1021 acttccggaa tcgggagcgc ggccgatgca aagtgccgat aaacataacg atctttgtag
1081 aaaccatcgg cgcagctatt taccgcagc acatatccac gccctcctac atcgaagctg
1141 aaagcacgag attcttcgcc ctccgagagc tgcacaggt cggagacgct gtcgaacttt
1201 tcgatcagaa acttctcgac agacgtcgcg gtgagttcag gctttttcat ggtggtcgtc
1261 tccttgtgag gggtcagggg cgtgggtcag gggatggtgg cggcaccggt cgtggcggcc
1321 gacctgcagg catgcaagct atcgaattcc tgcagcccgg gggatctggt gtaatttata
1381 atttatattt cttttcttaa taaataaata aatagtcaag tttatgttg agttttatga
1441 tttatatttt aagttatttc aactgcaaca ccagcaccac gacctacta cagcaaaaaa
1501 cgtacaagaa ggaagaagg aataaaaaga gtggtattct cttacaatat gttttatggc
1561 ataaaaggtg tggcattca tatcaaatat aaagtagtgt tgtttaacgt tacttttcta
1621 ggttgaatag tatattccaa cagatgatga ggggttccca atcctaaacc catttgccgt
1681 tcccagaagc atgaaaccac cacgcaccgg atcctctaga acaacaaca ttgcattcat
1741 tttatgtttc aggttcaggg ggaggtgtgg gaggtttttt aaagcaagta aaacctctac
1801 aatgttggtg tggtgatta tgatcagtcg acctgcaggc atgcaagctt ggcgtaatca
1861 tggcatagc tgtttcctgt gtgaaattgt tatccgctca caattccaca caacatacga
1921 gccggaagca taaagtgtaa agcctggggg gcctaagtag tgagctaact cacattaatt
1981 gcgttgcgct cactgcccgc tttccagtcg ggaaacctgt cgtgccagct gcattaatga
2041 atcggccaac gcgcggggag aggcggtttg cgtattgggc gctcttcgc ttcctcgctc
2101 actgactcgc tgcgctcggc cgttcggctg cggcgagcgg tatcagctca ctcaaaggcg
2161 gtaatacggc tatccacaga atcaggggat aacgcaggaa agaacatgtg agcaaaaggc
2221 cagcaaaagg ccaggaaccg taaaaggcc gcgttgctgg cgtttttcca taggctccgc
2281 cccctgacg agcatcaca aaatcgacgc tcaagtcaga ggtggcgaaa cccgacagga
2341 ctataaagat accaggcgtt tccccctgga agctccctcg tgcgctctcc tgttccgacc
2401 ctgccgctta ccggatacct gtccgccttt ctcccttcgg gaagcgtggc gctttctcat
2461 agctcacgct gtaggtatct cagttcgggt taggtcgttc gctccaagct gggctgtgtg
2521 cacgaacccc ccgttcagcc cgaccgctgc gccttatccg gtaactatcg tcttgagtcc
2581 aacccggtg gacacgactt atcgccactg gcagcagcca ctggtaacag gattagcaga
2641 gcgaggtatg taggcggtgc tacagagttc ttgaagtgtt ggcctaacta cggctacact
2701 agaagaacag tatttggtat ctgcgctctg ctgaagccag ttaccttcgg aaaaagagtt
2761 ggtagctctt gatccggcaa acaaacacc gctggtagcg gtggtttttt tgtttgcaag
2821 cagcagatta cgcgcagaaa aaaaggatct caagaagatc ctttgatctt ttctacgggg
2881 tctgacgctc agtgaacga aaactcacgt taagggattt tggatcatgag attatcaaaa
2941 aggatcttca ctagatcct tttaaattaa aatgaagtt taaatcaat ctaaagtata
3001 tatgagtaaa cttggtctga cagttacca tgcttaatca gtgaggcacc tatctcagcg
3061 atctgtctat ttcgttcatc catagttgcc tgactccccg tcgtgtagat aactacgata
```

3121 cgggagggct taccatctgg cccagtgct gcaatgatac cgcgagacc acgctcaccg  
3181 gctccagatt tatcagcaat aaaccagcca gccggaaggg ccgagcgag aagtggctct  
3241 gcaactttat ccgcctccat ccagtctatt aattgttgcc gggagctag agtaagtagt  
3301 tcgccagtta atagtttgcg caacgttggt gccattgcta caggcatcgt ggtgtcacgc  
3361 tcgtcgtttg gtatggcttc attcagctcc ggttcccaac gatcaaggcg agttacatga  
3421 tccccatgt tgtgcaaaaa agcggttagc tccttcggtc ctccgatcgt tgtcagaagt  
3481 aagttggccg cagtgttatc actcatgggt atggcagcac tgcataattc tcttactgtc  
3541 atgccatccg taagatgctt ttctgtgact ggtgagtact caaccaagtc attctgagaa  
3601 tagtgtatgc ggcgaccgag ttgctcttgcc ccggcgtaaa tacgggataa taccgcgcca  
3661 catagcagaa ctttaaaagt gctcatcatt ggaaaacggt ctccggggcg aaaactctca  
3721 aggatcttac cgctgttgag atccagttcg atgtaacca ctctgtcacc caactgatct  
3781 tcagcatctt ttactttcac cagcgtttct gggtgagcaa aaacaggaag gcaaaatgcc  
3841 gcaaaaaagg gaataagggc gacacggaaa tgttgaatac tcatactctt ctttttcaa  
3901 tattattgaa gcatttatca gggttattgt ctcatgagcg gatacatatt tgaatgtatt  
3961 tagaaaaata aacaaatagg ggttccgcgc acatttccc gaaaagtgcc acctgacgtc  
4021 taagaaacca ttattatcat gacattaacc tataaaaaata ggcgtatcac gaggcccttt  
4081 cgtctcgcgc gtttcggtga tgacggtgaa aacctctgac acatgcagct cccggagacg  
4141 gtcacagctt gtctgtaagc ggatgccggg agcagacaag cccgtcaggg cgcgtcagcg  
4201 ggtgttgccg ggtgtcgggg ctggcttaac tatgcggcat cagagcagat tgtactgaga  
4261 gtgcaccata tgcggtgta aataccgcac agatgcgtaa ggagaaaaata ccgcatcagg  
4321 cgccattcgc cattcaggct gcgcaactgt tgggaagggc gatcgggtcg ggcctcttcg  
4381 ctattacgcc agctggcgaa agggggatgt gctgcaaggc gattaagtg ggtaacgcca  
4441 gggttttccc agtcacgacg ttgtaaaacg acggccagtg aattaattcg ttgcaggaca  
4501 ggatgtggtg cccgatgtga ctagctcttt gctgcaggcc gtcctatcct ctggttccga  
4561 taagagacc agaactccgg cccccaccg cccaccgcca ccccataca tatgtggtac  
4621 gcaagtaaga gtgcctcgc atgccccatg tgccccacca agagctttgc atcccataca  
4681 agtccccaaa gtggagaacc gaaccaattc ttcgctggca gaacaaaagc ttctgcacac  
4741 gtctccactc gaatttggag ccggccggcg tgtgcaaaag aggtgaatcg aacgaaagac  
4801 ccgtgtgtaa agccgcgttt ccaaaatgta taaaaccgag agcatctggc caatgtgcat  
4861 cagttgtggt cagcagcaaa atcaagtga tcatctcagt gcaactaaag ggggatcta  
4921 gatcggggta ccgccaccAT GGGTAAGCCT ATCCCTAACC CTCTCCTCGG TCTCGATTCT  
4981 ACGACTAGTG GAGGAGGAGG TTCTGGTGGT GGTCACAACA ACTCCACAAA AACCAAGGAA  
5041 ATGTTTCATCG TGCGTGCTCT AGAAAAGATC CTTGCCGATA AGGACATACG GCGCTCCCAT  
5101 CACTCGCAGC TGAAGAAGTC CTGCGATTCT GCGCTGGAGC AGATTAAGGC GGAGCTAATC  
5161 AGTGCCGGCC AGATCGCAGA GGGCAATGAG CTGCCCTGTG CCGCACTCCC GCTGCCAAG  
5221 AATGATGCAG CGAGCATCAT AAATGCGGAG ACCTACTTTC TCCCCTTCGA GCTTGCCTGC

5281 AAGAGCCGCT CGCCCAGGAT CGTGGTCACC GCACTGGACT GCCTGCAGAA ACTCATTGCC  
5341 TATGGCCATT TGACAGGATC CATTGAGGAC TCGGCCAATC CGGGTCACCT GTCATCGAC  
5401 CGTATCGTTG TGACCATATA TGGCTGCTTC AGTGGTCCCC AGACGGACGA GGCCGTCCAA  
5461 CTGAGATAA TAAAGGCTCT GCTCACGGTG GTCACCTCGC AGCATGTGGA AATCCATGAA  
5521 TTCACACTGC TGCAAGCTGT GCGCACCTGC TACGACATCT ATTTGTCCAG CAAGAACCTG  
5581 GTCAATCAGA CCACAGCACG CGCTACGCTC ACCCAAATGT TGAACGTGAT ATTTGCCCCG  
5641 ATGGAGAATC AAGTGTACGA GCTACCACCT CCCAATTCCA ATCCCACCAA CGGCAGCATC  
5701 CACTCGGAGG ATTGCAATGG CTCGGGAGAG GAGTCGCTGC GGGATTCCGA CGAAGTAATT  
5761 GCCTCGGAAC TGCTGGCGGA GATCATATCA GCTGCCTACA ATGAGGCGAT GAAGGATCAG  
5821 GAATCGGTGC GTGAGCCAGA GCCAACACTT AATGGAAACG ACTACTCCTC GCACTCGGAT  
5881 CACGACAGTG TGGAGCTGCA CAGCGAAAAC GATGCGGTTG TAACGGCTAA GTTTACGCAC  
5941 ATCCTGCAGA AAGATGCTTT TCTCGTGTC CGGGCACTGT GCAAGCTATC GATGAAGCCT  
6001 TTGCCGGATG GACATCCAGA TCCGAAATCG CACGAGCTGC GTTCCAAGGT GCTGTCATTG  
6061 CATCTGCTGC TGCTCATCCT CCAGAATGCC GGGCCCGTCT TCCGCTCCAA CGAGATGTTC  
6121 ATCATGGCCA TTAAGCAGTA CCTGTGCGTG GCCTTGTC AAACACGGAGT CAGTCTGGTG  
6181 CCGGAGGTCT TCGAGCTGTC GCTTTCAATC TTCGTTGCC TACTCTCGAA CTTCAAGGTG  
6241 CATCTTAAGC GGCAGATAGA GGTGTTCTTC AAGGAAATCT TCCTAAACAT TCTTGAGGCG  
6301 AACTCAAGCA GCTTCGAGCA CAAATGGATG GTAATCCAAG CGCTGACACG TATTTGTGCT  
6361 GACGCCCAGT CCGTGGTGGG TATCTATGTT AATTACGATT GCGACTTTTC GGCTGCAAAC  
6421 CTTTTTGAGA GACTGGTCAA CGATCTTTCG AAAATTGCC AGGGTCGTCG GGCTCTCGAA  
6481 CTGGGCGCCA ATCCGATGCA AGAGAAATCG ATGCGCATTG GCGGCCTGGA GTGTCTTGTC  
6541 TCCATTCTTA AGTGCATGGT AGAGTGGAGT AAGGACTTGT ATGTTAATCC AAACATGCCG  
6601 GTTCCACCTA TGCAAGTCCA ATCGCCGACA AGCACTGAGC AGGATCAGGC GGACACAAC  
6661 ATCCAAACGA TGCACAGTGG TTCCAGTCAT AGTTTGAAC CCAATCAGGA GCAACTACAG  
6721 GATCTTCCCG AGGCATTGGA GGAGCGCAAG ATGCGCAAGG AAGTGTGGA AACAGGCATT  
6781 GAGTTATTCA ATCGTAAGCC TCAGAAAGGA GTGCAATTCC TGCAGGAGAA GCAGTTGCTG  
6841 GGTGCCACAT GCGGGGACAT TGCGCGCTGG CTGCACGAGG ACGAACGACT GGACAAGACA  
6901 GTGATCGGAA ACTACATTGG CGAGAATGAC GACCACTCCA AGGAAGTGAT GTGCGCTTAC  
6961 ATCGATGCCT TTGACTTTCG CCAAATGGAG GTGGTGGCCG CTTGAGATT TCTTCTCGAG  
7021 GGGTTCCGCC TGCCAGGAGA AGCACAAAAA ATCGATCGGC TGATGGAGAA GTTCGCCAGT  
7081 AGATACTGCG AATGCAATCC GAAGAACCAG CTATTCCAAA GCGCAGACAC CGTCTACGTG  
7141 CTGGCATTCA GCATCATTAT GCTGACCACG GATCTTCATT CGCCGAGGT CAAGCACAAAG  
7201 ATGACCAAGG AGCAGTACAT TAAAATGAAC CGCGGCATCA GCGACAGCAA GTCCGATTG  
7261 CCCGAGGAGT ACTTGTCGTC CATCTACGAC GAGATTTCTG AACACGAAAT TAAGATGAAG  
7321 AACAACTCCG GTATGCTTCA ACAGGCGAAA CCCACTGGAA AGCAGGCCTT CATAACGGAG  
7381 AAACGCAGAA AGCTGTTGTG GAACATGGAG ATGGAGGTCA TCTCGCTGAC GGCCACCAAT

7441 CTAATGCAGT CAGTTTCGCA CGTCAAGTCA CCCTTCACCT CAGCGAAACA CTTGGAGCAT  
7501 GTCCGGCCCA TGTTCAAAT GGCTTGGACA CCATTTCTGG CCGCTTCTC TGTGGGTCTC  
7561 CAGGACTGCG ACGATCCTGA GATTGCTACA CTCTGCTTGG ATGGTATACG TTGTGCTATT  
7621 CGAATCGCAT GCATCTTCCA CATGTCCCTG GAGCGAGATG CCTATGTACA AGCCCTGGCC  
7681 AGGTTTACTC TCCTGAATGC TAACTCGCCC ATCAACGAAA TGAAGGCCAA GAATATCGAT  
7741 ACCATCAAGA CGCTTATAAT GGTAGCCAC ACGGATGGCA ATTATCTGGG CAGCAGCTGG  
7801 CTGGATATAG TGAAGTGCAT TAGCCAGTTG GAGCTGGCCC AACTGATCGG CACTGGGGTG  
7861 CGGCCCCAGT TTCTTTCTGG AGCGCAGACA ACGCTCAAGG ACTCGCTTAA TCCCAGCGTG  
7921 AAAGAACACA TCGGCGAGAC GAGCAGCCAG AGCGTGGTGG TCGCAGTCGA TCGTATTTTC  
7981 ACCGGCTCAA TGCGACTGGA TGGCGATGCT ATCGTGGACT TCGTGAAGGC CCTGTGCCAG  
8041 GTGTCTGTGG ATGAGCTTCA GCAGCAGCAA CCGAGGATGT TCTCCTTGCA AAAGATAGTG  
8101 GAAATTAGTT ACTACAACAT GGAGCGTATT CGTCTGCAGT GGTCACGCAT TTGGCAAGTT  
8161 TTGGGTGAGC ACTTTAACGC GGTCGGATGC AATAGCAACG AGGAGATCTC ATTTTTCGCC  
8221 CTGGACTCAC TGCGTCAGTT GTCGATGAAG TTCATGGAGA AGGGCGAGTT CAGTAATTTT  
8281 CGCTTCCAGA AGGATTTCTT GCGTCCCTTT GAGCATATCA TGAAGAAAAA CGCATCGCCG  
8341 GCAATACGAG ATATGGTGGT GCGCTGCATT GCCCAGATGG TAAACTCACA GGCGCATAAC  
8401 ATCCGTTCCG GCTGGAAGAA TATCTTTAGC ATTTTCCACC TGGCAGCGGG AGACAACGAA  
8461 GAGCCAATTG TGGAGCTGGC CTTCAAACC ACGGGCAAAA TCATCGGTGA TCTGTACAAG  
8521 CGTCAGTTCG CCATTATGGT GGA CTGCTC CAGGATGCGG TCAAGTGCCT GTCAGAGTTC  
8581 GCCACCGCCA GATTCCCCGA TACCAGCATG GAAGCCATAC GTCTGGTCCG TACCTGCGCG  
8641 CAGTGCCTCC ACGAGGCACC ACAACTGTTT GCGGAGCATG CCGGCATGGA GAACGACGCC  
8701 TCGGTGGCCG AGGAGGATCG AGTCTGGGTG GCGGGCTGGT TTCCGATGCT ATTCTCGCTT  
8761 TCCTGCGTGG TCAATCGCTG CAAATTGGAT GTGCGTACTC GCGCCTTAAC CGTGTCTTTT  
8821 GAGATTGTGA AGACGTATGG TGAGAGCTTC AAGCCCCATT GGTGGAAGGA TCTCTTCAAT  
8881 GTGATCTTCC GTATCTTCGA CAACATGAAA TTGCCGGAGC ACGTCACCGA GAAGTCCGAA  
8941 TGGATGACGA CCACATGCAA CCACGCCTTG TACGCTATTA TTGATGTCTT CACGCAGTAT  
9001 TTCGATGTTT TTGGTCATCT GCTGCTGGAG GAGCTCTTCG CCCAGCTGCA TTGGTGTGTT  
9061 CAGCAGAGTA ACGAGCAGTT GGCGGATCT GGCACCAATT GCCTGGAGAA CCTCGTCATT  
9121 TCGAATGGAT TCAAGTTCAA CGAGTCCACC TGGACAAGA CGTGCCAGTG CATCCTGGAC  
9181 ATCTTCAACG CCACTTTGCC GCAGGATCTC CTCAGTTGGC GGCCGAAAGC ACATTCCAGT  
9241 AACAAATAC CCCAGGAGCA CAACCACTTT GAGGCGCTGC ATATCCGCTG CGTAGTCCAG  
9301 CTGGAAGTGA TACAGACCAT GGATAACATT GTCTTTTTCC CGGCCACGTC GCGCAAGGAG  
9361 GATGCCGAAA CGCTGGCCCA GGC GGCGCA GACTTAACAG GCGGCAGGAG CGGTTTCGAG  
9421 TCGCAGCTGC TGGAGTGCCA GCGGGAGGAG CAGGGAATGT ACGGCTATCT GAGAACCCGC  
9481 CAGCTGCTCA CCCTGGCCGA CTGTCTGATG CAGTCGCACC GTTTTGCCAA GCGCTTCAAC  
9541 GCCGATCACG ACCAACGCAG CCTGCTTTGG CGGCGGGAT TCAAGGGATC TGTTAAACCG



<p>9601 AATCTGCTGA AGCAGGAGAC CTCGTCGCTG GCCTGCGTCC TGCGCATTTT CTTCAAGATG            9661 TACGGCGACG AGAATAGACG CAGCGATTGG CCCGGCATCG AGCAGGAACT GGTGCAGGTC            9721 TGCAAGGAGG CACTGGGCTA CTATTTGAGT TTGCAGAGCG AGGCACACCG AGATGCGTGG            9781 ACATCGCTGC TGCTGCTCAT CCTGACGCGC CTGCTCAAGA TGTCCGATGC CAGGTTCCGC            9841 ACCCACGTTT CCAACTACTA CAGCCTGCTG TGCGAGATGA TGTGCTTCGA CCTCAAGCCC            9901 GAACTGAGAA GTGTCCTTAG GCGTGTGTTT ATGCGCATCG GTCCAGTATT CAATATAATG            9961 AGCGTTAAAT AAttctagtc gaccatgaag atcaagatca ttgccccgcc agagcgcgaag            10021 tactctgtct gggcccttcg aaGGTAAGCC TATCCCTAAC CCTCTCCTCG GTCTCGATTC            10081 TACGcgtacc ggtCATCATC ACCATCACCA TTGAgtttaa acccgctgat cagcctcgac            10141 tgtgccttct aagatccaga catgataaga tacattgatg agtttggaca aaccacaact            10201 agaatgcagt gaaaaaatg ctttatttgt gaaatttgtg atgctattgc tttatttcta            10261 accatt</p> <p>//</p>				
<p>pMT-hyg-V5-iRFP713-Sec71</p>				
LOCUS	pMT-hyg-V5-iRFP713-Sec71	11268 bp	ds-DNA	circular
<p>28-FEB-2020</p>				
DEFINITION	pMT-puro Sequencing Result			
ORGANISM	other sequences; artificial sequences; vectors.			
COMMENT	pMT-mAX2m-blast from 1 to 5500			
COMMENT	pMT-mAX2m-neo from 1 to 5813			
COMMENT	pMT-mAPEX2m from 1 to 5561			
COMMENT	pMT-mSOG1m from 1 to 5078			
COMMENT	pMT-mSOG4m from 1 to 5114			
COMMENT	ApEinfo:methylated:0			
FEATURES	Location/Qualifiers			
misc_feature	4892..4909	<p>/label=Metallothionein_primer            /ApEinfo_fwdcolor=#ff3600            /ApEinfo_revcolor=#ff3600            /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}            width 5 offset 0</p>		
misc_feature	5995..6015	<p>/label=Linker GL3            /ApEinfo_fwdcolor=#ffcc66            /ApEinfo_revcolor=#00ff00            /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}</p>		

```
width 5 offset 0
misc_feature 4927..4932
  /label=KpnI
  /ApEinfo_fwdcolor=#ffffcc
  /ApEinfo_revcolor=#ffffcc
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature 5992..5994
  /label=Linker GL3(1)
  /ApEinfo_label=Linker GL3
  /ApEinfo_fwdcolor=#ffcc66
  /ApEinfo_revcolor=#00ff00
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature 8152..8154
  /label=S191
  /ApEinfo_fwdcolor=#66ff66
  /ApEinfo_revcolor=#00ff00
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
CDS 5035..5982
  /codon_start=1
  /product="phytochrome-based near-infrared fluorescent
protein, originally termed iRFP (Filonov et al., 2011)"
  /note="name was changed to iRFP713 (Shcherbakova and
Verkhusha, 2013)"

/translation="MAEGSVARQPDLLTCDDEPIHIPGAIQPHGLLLALAADMTIVAG
S
DNLPELTGLAIGALIGRSAADVDFSETHNRLTIALAEPGAAVGAPITVGFTMRKDAGF
I
GSWHRHDQLIFLELEPPQRDVAEPQAFFRRTNSAIRRLQAAETLESACAAAAQEVRKI
T
```

```
GFDRVMIYRFASDFSGEVIAEDRCAEVESKLGHLHYPASTVPAQARRLYTINPVRIIPD
I
NYRPVPTPDLNPVTGRPIDLFAILRSVSPVHLEFMRNIGMHGTMSISILRGERLWG
L IVCHHRTPYYVDLDGRQACELVAQVLAWQIGVMEE"
/label=iRFP713
/ApEinfo_fwdcolor=#9a69fe
/ApEinfo_revcolor=#cde7f7
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 10326..10326
/label=T>C silent
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 4942..4983
/label=V5
/ApEinfo_fwdcolor=#ffffcc
/ApEinfo_revcolor=#ffffcc
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind complement(10226..10247)
/label=Chang-R1
/ApEinfo_fwdcolor=#fb53d0
/ApEinfo_revcolor=#fc5a5d
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 4984..4989
/label=SpeI
/ApEinfo_fwdcolor=#ffffcc
/ApEinfo_revcolor=#ffffcc
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind 6926..6945
/label=sec71-GF4
```

```

/ApEinfo_fwdcolor=#ff00bd
/ApEinfo_revcolor=#ff0003
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 4990..5013
/label=Linker GL3(2)
/ApEinfo_label=Linker GL3
/ApEinfo_fwdcolor=#ffcc66
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 8041..8043
/label=E677 (E740 in garz)
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
regulatory 5029..5038
/regulatory_class="other"
/note="vertebrate consensus sequence for strong
initiation
of translation (Kozak, 1987)"
/label=vertebrate consensus sequence for strong
initiation
of translation (Kozak, 1987)
/ApEinfo_fwdcolor=pink
/ApEinfo_revcolor=pink
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 7602..7602
/label=G>A silent
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature complement(225..1250)
```

```

        /label=Hyg
        /ApEinfo_fwdcolor=#ffffcc
        /ApEinfo_revcolor=#00ff00
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
gene      complement(143..173)
        /gene="puro (variant)"

/translation="MGSCAPFGRALRVVGRASGTGLAGHAPGARSFGHLDVGGDGEAE
PLVEGEVAGRGGLEGGHPGALGRLHSGEHDGAAQTLALVVRDADGGQEPRGLLGPV
RRQEAFHLLLRGQPGTAQLGHARADLGEHRPRFDALRRGPDRHRGAVVRDPHLADVEP
DAREEEFLQLGDPLDVAVRVDGVARGGVVGERGGEGAYGPGDVVAGGEAHRGLVLGHG
        RSSPCEGSAWVRGWRRHSWRPTCRHASYPARAARGICCNL*"
        /label=puro (variant)
        /ApEinfo_fwdcolor=#ffffcc
        /ApEinfo_revcolor=#ffffcc
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
misc_feature 95..115
        /label=New Feature
        /ApEinfo_fwdcolor=cyan
        /ApEinfo_revcolor=#00ff00
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
promoter  4463..4479
        /label=M13_forward20_primer
        /ApEinfo_fwdcolor=#ccffed
        /ApEinfo_revcolor=#ccffed
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
misc_feature 4448..4470
        /label=M13_pUC_fwd_primer
        /ApEinfo_fwdcolor=#ff3600
```

```

    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
promoter    complement(3926..3954)
    /label=AmpR_promoter
    /ApEinfo_fwdcolor=#ccffed
    /ApEinfo_revcolor=#ccffed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
rep_origin  complement(2250..2869)

/translation="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGY
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLTTIGGPKELTAFLHNMGDHVTRL
DRWEPELNEAIPNDERDTMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPL
LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
    EIGASLIKHW*"
    /label=pBR322_origin
    /ApEinfo_fwdcolor=pink
    /ApEinfo_revcolor=pink
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
CDS        6016..10971
    /label=Sec71
    /ApEinfo_fwdcolor=#99ccff
    /ApEinfo_revcolor=#cde7f7
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature complement(1876..1898)

/translation="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGY
```

```
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE

YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLTTIGGPKELTAFLHNMGDHSVTRL

DRWEPELNEAIPNDERDTTMPVAMATTLRKLTLGELLTLASRQQLIDWMEADKVAGPL

LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
    EIGASLIKHW*"
    /label=M13_pUC_rev_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    complement(1350..1708)
    /label=Copia Promoter?
    /ApEinfo_fwdcolor=#cde7f7
    /ApEinfo_revcolor=#cde7f7
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    10569..10569
    /label=T>G silent
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    complement(4327..4482)
    /label=lacZ_a
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    complement(4113..4135)
    /label=pGEX_3_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
```

```
width 5 offset 0
primer_bind    8772..8791
               /label=sec71-GF6
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
gene           complement(3024..3884)
               /gene="Ampicillin"
               /label=Ampicillin
               /ApEinfo_fwdcolor=#ffffcc
               /ApEinfo_revcolor=#ffffcc
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
promoter       complement(1912..1941)

/translation="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGY
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHVTRL
DRWEPELNEAIPNDERDTMPVAMATTLRKLTLGELLTLASRQQLIDWMEADKVAGPL
LRSALPAGWFIADKSGAGERSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
EIGASLIKHW*"
               /label=lac_promoter
               /ApEinfo_fwdcolor=#ccffed
               /ApEinfo_revcolor=#ccffed
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature   8631..8631
               /label=A>G in 40A
               /ApEinfo_fwdcolor=cyan
               /ApEinfo_revcolor=#00ff00
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
```



```
width 5 offset 0
promoter complement(1859..1877)

/translation="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGY
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHVTRL
DRWEPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPL
LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
EIGASLIKHW*"
/label=M13_reverse_primer
/ApEinfo_fwdcolor=#ccffed
/ApEinfo_revcolor=#ccffed
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 4489..4856
/label=MT-promoter
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
protein_bind 7735..8307
/label=Sec7 domain
/ApEinfo_fwdcolor=#0080ff
/ApEinfo_revcolor=#0080ff
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
CDS complement(4323..4391)
/label=LacZ alpha
/ApEinfo_fwdcolor=#6495ed
/ApEinfo_revcolor=#6495ed
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
```

```
primer_bind    3998..4015
               /label=pQE60-F
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
misc_feature   10554..10554
               /label=C>T silent
               /ApEinfo_fwdcolor=cyan
               /ApEinfo_revcolor=#00ff00
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
primer_bind    complement(1859..1879)
               /label=M13R_GW
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
primer_bind    complement(1859..1879)
               /label=M13-rev
               /ApEinfo_fwdcolor=cyan
               /ApEinfo_revcolor=green
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
primer_bind    8252..8275
               /label=Sec71-DRSC01893-F (8A10)
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
primer_bind    4462..4479
               /label=M13-fwd
               /ApEinfo_fwdcolor=cyan
               /ApEinfo_revcolor=green
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
```

```
primer_bind    4436..4459
               /label=M13F
               /ApEinfo_fwdcolor=#0a00ff
               /ApEinfo_revcolor=#0a00ff
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
primer_bind    10056..10075
               /label=sec71-GF8
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
primer_bind    4832..4849
               /label=SEQ-MT-F2
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
rep_origin     complement(2247..2929)
               /label=ColE1 origin
               /ApEinfo_fwdcolor=gray50
               /ApEinfo_revcolor=gray50
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
primer_bind    9511..9532
               /label=sec71-GF7
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
CDS            complement(3027..3686)
               /label=AmpR
               /ApEinfo_fwdcolor=yellow
               /ApEinfo_revcolor=yellow
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
```

```
misc_binding    complement(1885..1907)
                /label=Lac0
                /ApEinfo_fwdcolor=#6495ed
                /ApEinfo_revcolor=#6495ed
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
primer_bind     complement(9564..9585)
                /label=Sec71-GR3
                /ApEinfo_fwdcolor=#ff00bd
                /ApEinfo_revcolor=#ff0003
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
primer_bind     complement(3605..3629)
                /label=Amp-GF
                /ApEinfo_fwdcolor=#ff00bd
                /ApEinfo_revcolor=#ff0003
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
primer_bind     3147..3170
                /label=Amp-GR1
                /ApEinfo_fwdcolor=#ff00bd
                /ApEinfo_revcolor=#ff0003
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
misc_feature    7071..7071
                /label=C>T confirmed in 40A 2013
                /ApEinfo_fwdcolor=cyan
                /ApEinfo_revcolor=#00ff00
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
primer_bind     4462..4479
                /label=M13F_GW
                /ApEinfo_fwdcolor=#ff00bd
                /ApEinfo_revcolor=#ff0003
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
```

```
primer_bind    1737..1759
               /label=Seq-EB-R
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
misc_feature   8559..8559
               /label=T>C in 40A
               /ApEinfo_fwdcolor=cyan
               /ApEinfo_revcolor=#00ff00
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
misc_feature   1254..1268
               /label=New Feature(1)
               /ApEinfo_label=New Feature
               /ApEinfo_fwdcolor=cyan
               /ApEinfo_revcolor=#00ff00
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
misc_feature   8670..8670
               /label=A>C in 40A
               /ApEinfo_fwdcolor=cyan
               /ApEinfo_revcolor=#00ff00
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
primer_bind    8199..8217
               /label=Sec71-GF11
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
primer_bind    7799..7822
               /label=Sec71-GF12
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
```

```
width 5 offset 0
misc_feature 8161..8163
  /label=M717(M194 in sec7 domain M>L = BFA-resistant)
  /label=M717L(BFA-resistant)
  /ApEinfo_fwdcolor=cyan
  /ApEinfo_revcolor=#00ff00
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind 7905..7927
  /label=Sec71-GF14
  /ApEinfo_fwdcolor=#ff00bd
  /ApEinfo_revcolor=#ff0003
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature complement(8101..8123)
  /label=Sec71-crRNA7(Protospacer)
  /ApEinfo_fwdcolor=#ccff66
  /ApEinfo_revcolor=#ffff9f
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 8190..8209
  /label=Sec71-crRNA8 Protospacer
  /ApEinfo_fwdcolor=#ccff66
  /ApEinfo_revcolor=#ffffcc
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 8830..8830
  /label=T>C Val>Ala in FRT40A
  /ApEinfo_fwdcolor=#ccff66
  /ApEinfo_revcolor=#00ff00
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
CDS 8892..8892
  /label=Sec71-PA
  /ApEinfo_fwdcolor=#99ccff
  /ApEinfo_revcolor=#cde7f7
```

```

    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
CDS      6979..6981
    /label=Sec71-PA(1)
    /ApEinfo_label=Sec71-PA
    /ApEinfo_fwdcolor=#99ccff
    /ApEinfo_revcolor=#cde7f7
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature  10365..10365
    /label=T>C silent(1)
    /ApEinfo_label=T>C silent
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature  9511..9532
    /label=Chang-F1
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind  6570..6589
    /label=sec71-GF3
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature  8892..8892
    /label=A>C silent
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
CDS      7071..7071
    /label=Sec71-PA(2)
```

```

    /ApEinfo_label=Sec71-PA
    /ApEinfo_fwdcolor=#99ccff
    /ApEinfo_revcolor=#cde7f7
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
CDS      7602..7602
    /label=Sec71-PA(3)
    /ApEinfo_label=Sec71-PA
    /ApEinfo_fwdcolor=#99ccff
    /ApEinfo_revcolor=#cde7f7
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature  10449..10449
    /label=G>A silent(1)
    /ApEinfo_label=G>A silent
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature  10753..10753
    /label=T>C silent(2)
    /ApEinfo_label=T>C silent
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind  7552..7571
    /label=Sec71-GF5
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind  complement(8728..8763)
    /label=Sec71-DRSC01893-R
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
```



```

    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    8149..8151
    /label=F190 F190Y=BFA-hypersensitive
    /label=F713Y(BFA-sensitive)
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    8721..8721
    /label=A>G 40A
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    10338..10338
    /label=T>C in 40A(1)
    /ApEinfo_label=T>C in 40A
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    6042..6059
    /label=GF9
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    complement(8377..8400)
    /label=Sec71-GR4
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    9139..9157
    /label=Sec71-GF13
```

```
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature    10972..10974
    /label=STOP
    /ApEinfo_fwdcolor=#66ccff
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind    8075..8096
    /label=Sec71-GF15
    /ApEinfo_fwdcolor=#fb53d0
    /ApEinfo_revcolor=#fc5a5d
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature    8101..8103
    /label=PAM
    /ApEinfo_fwdcolor=#fc81f0
    /ApEinfo_revcolor=#fc81f0
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature    8210..8212
    /label=PAM(1)
    /ApEinfo_label=PAM
    /ApEinfo_fwdcolor=#fc81f0
    /ApEinfo_revcolor=#fc81f0
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature    complement(11179..11198)
    /label=EBV_rev_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature    complement(11136..11153)
```

```
    /label=BGH_rev_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature 11096..11113
    /label=6xHis
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature 11045..11086
    /label=V5(1)
    /ApEinfo_label=V5
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature 11013..11032
    /label=New Feature(2)
    /ApEinfo_label=New Feature
    /ApEinfo_fwdcolor=#ffffcc
    /ApEinfo_revcolor=#ffffcc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature 11033..11038
    /label=ApaI GGGCC^C
    /ApEinfo_fwdcolor=#ffffcc
    /ApEinfo_revcolor=#ffffcc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind 10664..10681
    /label=Sec71-GF10
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
```

width 5 offset 0

ORIGIN

```
1 ataagctgca ataaacaagt tctagagtcg gtgggcctcg ggggcgggtg cggggtcggc
61 ggggccgccc cgggtggcctt cggtcggagc catggggtcg tgcgctcctt tcggtcgggc
121 gctgcgggtc gtggggcggg cgtcaggcac cgggcttgcg ggtcatgcac cagttgctgg
181 ctgaggcggtt ctcgaaatca gctcttgttc ggtcggcatc tactctattc ctttgcctc
241 ggacgagtg cggggcgctg gtttccacta tcggcgagta cttctacaca gccatcggtc
301 cagacggccg cgcttctgcg ggcgatttgt gtacgcccga cagtcccggc tccggatcgg
361 acgattgcgt cgcacgcacc ctgcgcccga gctgcatcat cgaaattgcc gtcaaccaag
421 ctctgataga gttggtcaag accaatgcgg agcatatacg cccggagccg cggcgatcct
481 gcaagctccg gatgcctccg ctcgaagtag cgcgtctgct gctccataca agccaaccac
541 ggcctccaga agaagatggt ggcgacctcg tattgggaat ccccgaacat cgcctcgtc
601 cagtcaatga ccgctgttat gcggccattg tccgtcagga cattgttggg gccgaaatcc
661 gcgtgcacga ggtgccggac ttcggggcag tcctcggccc aaagcatcag ctcatcgaga
721 gcctgcgcga cggacgcact gacggtgctg tccatcacag tttgccagtg atacacatgg
781 ggatcagcaa tcgcgcatat gaaatcacgc catgtagtgt attgaccgat tccttgcggt
841 ccgaatgggc cgaacccgct cgtctggcta agatcggccg cagcgatcgc atccatggcc
901 tccgcgaccg gctgcagaac agcgggcagt tcggtttcag gcaggctctg caacgtgaca
961 ccctgtgcac ggcgggagat gcaataggtc aggctctcgc tgaattcccc aatgtcaagc
1021 acttccggaa tcgggagcgc ggccgatgca aagtgccgat aaacataacg atctttgtag
1081 aaaccatcgg cgcagctatt taccgcagg acatatccac gccctctac atcgaagctg
1141 aaagcacgag attcttcgcc ctccgagagc tgcacaggt cggagacgct gtcgaacttt
1201 tcgatcagaa acttctcgac agacgtcgcg gtgagttcag gctttttcat ggtggtcgtc
1261 tccttgtgag gggtcagggg cgtgggtcag gggatggtgg cggcaccggt cgtggcggcc
1321 gacctgcagg catgcaagct atcgaattcc tgcagcccgg gggatctggt gtaatttata
1381 atttatattt ctttcttaa taaataaata aatagtcaag tttatgttg agttttatga
1441 tttatatttt aagttatttc aactgcaaca ccagcaccac gacctacta cagcaaaaaa
1501 cgtacaagaa ggaaagaagg aataaaaaga gtggtattct cttacaatat gttttatggc
1561 ataaaaggtg tggcattca tatcaaatat aaagtagtgt tgtttaacgt tacttttcta
1621 ggttgaatag tatattccaa cagatgatga ggggttccca atcctaaacc catttgccgt
1681 tcccagaagc atgaaaccac cacgcaccgg atcctctaga acaacaaca ttgcattcat
1741 tttatgtttc aggttcaggg ggaggtgtgg gaggtttttt aaagcaagta aaacctctac
1801 aatgtggta tggctgatta tgatcagtcg acctgcaggc atgcaagctt ggcgtaatca
1861 tggatcatagc tgtttcctgt gtgaaattgt tatccgctca caattccaca caacatacga
1921 gccggaagca taaagtgtaa agcctggggg gcctaagtag tgagctaact cacattaatt
1981 gcgttgcgct cactgcccgc tttccagtcg ggaacctgt cgtgccagct gcattaatga
```

2041 atcggccaac gcgcggggag aggcggtttg cgtattgggc gctcttccgc ttcctcgcctc  
2101 actgactcgc tgcgctcggc cgttcggctg cggcgagcgg tatcagctca ctcaaaggcg  
2161 gtaatacggc tatccacaga atcaggggat aacgcaggaa agaacatgtg agcaaaaggc  
2221 cagcaaaagg ccaggaaccg taaaaaggcc gcgttgctgg cgtttttcca taggctccgc  
2281 cccctgacg agcatcacia aaatcgacgc tcaagtcaga ggtggcgaaa cccgacagga  
2341 ctataaagat accaggcgtt tccccctgga agctccctcg tgcgctctcc tgttccgacc  
2401 ctgccgctta ccggatacct gtccgccttt ctcccttcgg gaagcgtggc gctttctcat  
2461 agctcacgct gtaggtatct cagttcgggt taggtcgttc gctccaagct gggctgtgtg  
2521 cacgaacccc ccgttcagcc cgaccgctgc gccttatccg gtaactatcg tcttgagtcc  
2581 aacccggtaa gacacgactt atcgccactg gcagcagcca ctggtaacag gattagcaga  
2641 gcgaggtatg taggcggtgc tacagagttc ttgaagtggc ggcctaacta cggctacact  
2701 agaagaacag tatttggtat ctgcgctctg ctgaagccag ttaccttcgg aaaaagagtt  
2761 ggtagctctt gatccggcaa acaaaccacc gctggtagcg gtggtttttt tgtttgcaag  
2821 cagcagatta cgcgcagaaa aaaaggatct caagaagatc ctttgatctt ttctacgggg  
2881 tctgacgctc agtggaacga aaactcacgt taagggattt tggatcatgag attatcaaaa  
2941 aggatcttca cctagatcct tttaaattaa aaatgaagtt ttaaatcaat ctaaagtata  
3001 tatgagtaaa cttggtctga cagttacca tgcttaatca gtgaggcacc tatctcagcg  
3061 atctgtctat ttcgttcatc catagttgcc tgactccccg tcgtgtagat aactacgata  
3121 cgggagggct taccatctgg cccagtgct gcaatgatac cgcgagacc acgctcaccg  
3181 gctccagatt tatcagcaat aaaccagcca gccggaaggc ccgagcgag aagtggctct  
3241 gcaactttat ccgcctccat ccagtctatt aattggtgcc gggagactag agtaagtagt  
3301 tcgccagtta atagtttgcg caacgttggt gccattgcta caggcatcgt ggtgtcacgc  
3361 tcgtcgtttg gtatggcttc attcagctcc ggttcccaac gatcaaggcg agttacatga  
3421 tccccatgt tgtgcaaaaa agcggttagc tccttcggtc ctccgatcgt tgtcagaagt  
3481 aagttggccg cagtgttatc actcatggtt atggcagcac tgcataattc tcttactgtc  
3541 atgccatccg taagatgctt ttctgtgact ggtgagtact caaccaagtc attctgagaa  
3601 tagtgtatgc ggcgaccgag ttgctcttgc ccggcgtcaa tacgggataa taccgcgcca  
3661 catagcagaa ctttaaaagt gctcatcatt ggaaaacggt cttcggggcg aaaactctca  
3721 aggatcttac cgctggtgag atccagttcg atgtaacca ctctgtcacc caactgatct  
3781 tcagcatctt ttactttcac cagcgtttct gggtgagcaa aaacaggaag gcaaaatgcc  
3841 gcaaaaaagg gaataagggc gacacggaaa tgttgaatac tcatactctt ctttttcaa  
3901 tattattgaa gcatttatca gggttattgt ctcatgagcg gatacatatt tgaatgtatt  
3961 tagaaaaata acaaatagg ggttccgcgc acatttccc gaaaagtgcc acctgacgtc  
4021 taagaaacca ttattatcat gacattaacc tataaaaaata ggcgatcac gaggcccttt  
4081 cgtctcgcgc gtttcggtga tgacggtgaa aacctctgac acatgcagct cccggagacg  
4141 gtcacagctt gtctgtaagc ggatgccggg agcagacaag cccgtcaggg cgcgtcagcg

```
4201 ggtgttggcg ggtgtcgggg ctggcttaac tatgcggcat cagagcagat tgtactgaga
4261 gtgaccata tgcggtgtga aataccgcac agatgcgtaa ggagaaaata ccgcatcagg
4321 cgccattcgc cattcaggct gcgcaactgt tgggaagggc gatcgggtgcg ggcctcttcg
4381 ctattacgcc agctggcgaa agggggatgt gctgcaaggc gattaagtgt ggtaacgcca
4441 gggttttccc agtcacgacg ttgtaaacg acggccagtg aattaattcg ttgcaggaca
4501 ggatgtggtg cccgatgtga ctagctcttt gctgcaggcc gtcctatcct ctggttccga
4561 taagagacc agaactccgg cccccaccg cccaccgcca ccccatata tatgtggtac
4621 gcaagtaaga gtgcctgcgc atgccccatg tgccccacca agagctttgc atcccatata
4681 agtccccaaa gtggagaacc gaaccaattc ttcgcgggca gaacaaaagc ttctgcacac
4741 gtctccactc gaatttggag ccggccggcg tgtgcaaaag aggtgaatcg aacgaaagac
4801 ccgtgtgtaa agccgcgttt ccaaatgta taaaaccgag agcatctggc caatgtgcat
4861 cagttgtggt cagcagcaaa atcaagtga tcatctcagt gcaactaaag gggggatcta
4921 gatcggggta ccgccaccAT GGGTAAGCCT ATCCCTAACC CTCTCCTCGG TCTCGATTCT
4981 ACGACTAGTG GAGGAGGAGG TTCTGGTGGT GGTGCGGCCG CATCTGCCG CACCATGGCT
5041 GAAGGATCCG TCGCCAGGCA GCCTGACCTC TTGACCTGCG ACGATGAGCC GATCCATATC
5101 CCCGGTGCCA TCCAACCGCA TGGACTGCTG CTCGCCCTCG CCGCCGACAT GACGATCGTT
5161 GCCGGCAGCG ACAACCTTCC CGAACTCACC GGA CTGGCGA TCGGCGCCCT GATCGGCCG
5221 TCTGCGGCCG ATGTCTTCGA CTCGGAGACG CACAACCGTC TGACGATCGC CTTGGCCGAG
5281 CCCGGGGCGG CCGTCGGAGC ACCGATCACT GTCGGCTTCA CGATGCGAAA GGACGCAGGC
5341 TTCATCGGCT CCTGGCATCG CCATGATCAG CTCATCTTCC TCGAGCTCGA GCCTCCCCAG
5401 CGGGACGTCG CCGAGCCGCA GCGTTCCTC CGCCGCACCA ACAGCGCCAT CCGCCGCTG
5461 CAGGCCGCCG AAACCTTGA AAGCGCTGC GCCGCCGCG CGCAAGAGGT GCGGAAGATT
5521 ACCGGCTTCG ATCGGGTGAT GATCTATCGC TTCGCCTCCG ACTTCAGCGG CGAAGTGATC
5581 GCAGAGGATC GGTGCGCCGA GGTCGAGTCA AA ACTAGGCC TGACTATCC TGCCTCAACC
5641 GTGCCGGCGC AGGCCGTCG GCTCTATACC ATCAACCCGG TACGGATCAT TCCCGATATC
5701 AATTATCGGC CGGTGCCGCT CACCCAGAC CTCAATCCGG TCACCGGGCG GCCGATTGAT
5761 CTTAGCTTCG CCATCCTGCG CAGCGTCTCG CCCGTCCATC TGAATTCAT GCGCAACATA
5821 GGCATGCACG GCACGATGTC GATCTCGATT TTGCGCGGCG AGCGACTGTG GGGATTGATC
5881 GTTTGCCATC ACCGAACGCC G TACTACGTC GATCTCGATG GCCGCCAAGC CTGCGAGCTA
5941 GTCGCCAGG TTCTGGCCTG GCAGATCGGC GTGATGGAAG AGggcgcgcc tggaggagga
6001 ggttctggtg gtggtCACAA CAACTCCACA AAAACCAAGG AAATGTTCAT CGTGCGTGCT
6061 CTAGAAAAGA TCCTTGCCGA TAAGGACATA CGGCGCTCCC ATCACTCGCA GCTGAAGAAG
6121 TCCTGCGATT CGGCGCTGGA GCAGATTAAG GCGGAGCTAA TCAGTGCCGG CCAGATCGCA
6181 GAGGGCAATG AGCTGCCCTG TGCCGCACTC CCGCTGCCA AGAATGATGC AGCGAGCATC
6241 ATAAATGCGG AGACCTACTT TCTCCCCTTC GAGCTTGCTT GCAAGAGCCG CTCGCCAGG
6301 ATCGTGGTCA CCGCACTGGA CTGCTGCAG AA ACTCATTG CCTATGGCCA TTTGACAGGA
```

6361 TCCATTCAGG ACTCGGCCAA TCCGGGTCAC CTGCTCATCG ACCGTATCGT TGTGACCATA  
6421 TATGGCTGCT TCAGTGGTCC CCAGACGGAC GAGGCCGTCC AACTGCAGAT AATAAAGGCT  
6481 CTGCTCACGG TGGTCACCTC GCAGCATGTG GAAATCCATG AATTCACACT GCTGCAAGCT  
6541 GTGCGCACCT GCTACGACAT CTATTTGTCC AGCAAGAACC TGGTCAATCA GACCACAGCA  
6601 CGCGCTACGC TCACCCAAAT GTTGAACGTG ATATTTGCCG GCATGGAGAA TCAAGTGAC  
6661 GAGTACCAC CTCCCAATTC CAATCCCACC AACGGCAGCA TCCACTCGGA GGATTGCAAT  
6721 GGCTCGGGAG AGGAGTCGCT GCGGGATTCC GACGAAGTAA TTGCCTCGGA ACTGCTGGCG  
6781 GAGATCATAT CAGCTGCCTA CAATGAGGCG ATGAAGGATC AGGAATCGGT CGGTGAGCCA  
6841 GAGCCAACAC TTAATGGAAA CGACTACTCC TCGCACTCGG ATCACGACAG TGTGGAGCTG  
6901 CACAGCGAAA ACGATGCGGT TGTAACGGCT AAGTTTACGC ACATCCTGCA GAAAGATGCT  
6961 TTTCTCGTGT TCCGGGCACT GTGCAAGCTA TCGATGAAGC CTTTGCCGGA TGGACATCCA  
7021 GATCCGAAAT CGCACGAGCT GCGTTCCAAG GTGCTGTCAT TGCATCTGCT GCTGCTCATC  
7081 CTCCAGAATG CCGGGCCCGT CTTCCGCTCC AACGAGATGT TCATCATGGC CATTAAAGCAG  
7141 TACCTGTGCG TGGCCTTGTC AAACAACGGA GTCAGTCTGG TGCCGGAGGT CTTGAGCTG  
7201 TCGCTTTCAA TCTTCGTTGC CCTACTCTCG AACTTCAAGG TGCATCTTAA GCGGCAGATA  
7261 GAGGTGTTCT TCAAGGAAAT CTTCTAAAC ATTCTTGAGG CGAACTCAAG CAGCTTCGAG  
7321 CACAAATGGA TGGTAATCCA AGCGCTGACA CGTATTTGTG CTGACGCCCA GTCCGTGGTG  
7381 GATATCTATG TTAATTACGA TTGCGACTTT TCGGCTGCAA ACCTTTTTGA GAGACTGGTC  
7441 AACGATCTTT CGAAAATTGC CCAGGGTCGT CAGGCTCTCG AACTGGGCGC CAATCCGATG  
7501 CAAGAGAAAT CGATGCGCAT TCGCGGCCTG GAGTGTCTTG TCTCCATTCT TAAGTGCATG  
7561 GTAGAGTGGG GTAAGGACTT GTATGTTAAT CCAAACATGC CGGTTCCACC TATGCAAGTC  
7621 CAATCGCCGA CAAGCACTGA GCAGGATCAG GCGGACACAA CTATCCAAAC GATGCACAGT  
7681 GGTTCAGTC ATAGTTTGAA CTCCAATCAG GAGCAACTAC AGGATCTTCC CGAGGCATTG  
7741 GAGGAGCGCA AGATGCGCAA GGAAGTGATG GAAACAGGCA TTGAGTTATT CAATCGTAAG  
7801 CCTCAGAAAG GAGTGCAATT CCTGCAGGAG AAGCAGTTGC TGGGTGCCAC ATGCGGGGAC  
7861 ATTGCGCGCT GGCTGCACGA GGACGAACGA CTGGACAAGA CAGTGATCGG AAACACTATT  
7921 GGCGAGAATG ACGACCACTC CAAGGAAGTG ATGTGCGCTT ACATCGATGC CTTTGACTTT  
7981 CGCCAAATGG AGGTGGTGGC CGCCTTGAGA TTTCTTCTCG AGGGGTTCCG CTGCCAGGA  
8041 GAAGCACAAA AAATCGATCG GCTGATGGAG AAGTTCGCCA GTAGATACTG CGAATGCAAT  
8101 CCGAAGAACC AGCTATTCCA AAGCGCAGAC ACCGTCTACG TGCTGGCATT CAGCATCATT  
8161 ATGCTGACCA CGGATCTTCA TTCGCCGAG GTCAAGCACA AGATGACCAA GGAGCAGTAC  
8221 ATTAATAATGA ACCGCGGCAT CAGCGACAGC AAGTCCGATT TGCCCCGAGGA GTACTTGTCG  
8281 TCCATCTACG ACGAGATTTT TGAACACGAA ATTAAGATGA AGAACAACCT CGGTATGCTT  
8341 CAACAGGCGA AACCCACTGG AAAGCAGGCC TTCATAACGG AGAAACGCAG AAAGCTGTTG  
8401 TGGAAATGG AGATGGAGGT CATCTCGCTG ACGGCCACCA ATCTAATGCA GTCAGTTTCG  
8461 CACGTCAAGT CACCCTTCAC CTCAGCGAAA CACTTGGAGC ATGTCCGGCC CATGTTCAA

8521 ATGGCTTGGG CACCATTCTT GCGCGCTTTC TCTGTGGGTC TCCAGGACTG CGACGATCCT  
8581 GAGATTGCTA CACTCTGCTT GGATGGTATA CGTTGTGCTA TTCGAATCGC ATGCATCTTC  
8641 CACATGTCCC TGGAGCGAGA TGCCTATGTA CAAGCCCTGG CCAGGTTTAC TCTCCTGAAT  
8701 GCTAACTCGC CCATCAACGA AATGAAGGCC AAGAATATCG ATACCATCAA GACGCTTATA  
8761 ATGGTAGCCC ACACGGATGG CAATTATCTG GGCAGCAGCT GGCTGGATAT AGTGAAGTGC  
8821 ATTAGCCAGT TGGAGCTGGC CCAACTGATC GGCAGTGGGG TCGGGCCCCA GTTCTTTTCT  
8881 GGAGCGCAGA CAACGCTCAA GGAICTGCTT AATCCCAGCG TGAAAGAACA CATCGGCGAG  
8941 ACGAGCAGCC AGAGCGTGGT GGTCGCAGTC GATCGTATTT TCACCGGCTC AATGCGACTG  
9001 GATGGCGATG CTATCGTGGG CTTCTGTAAG GCCCTGTGCC AGGTGTCTGT GGATGAGCTT  
9061 CAGCAGCAGC AACCGAGGAT GTTCTCCTTG CAAAAGATAG TGGAAATTAG TTACTIONAAC  
9121 ATGGAGCGTA TTCGTCTGCA GTGGTCACGC ATTTGGCAAG TTTTGGGTGA GCACTTTAAC  
9181 GCGGTCGGAT GCAATAGCAA CGAGGAGATC TCATTTTTCG CCCTGGACTC ACTGCGTCAG  
9241 TTGTGCATGA AGTTCATGGA GAAGGGCGAG TTCAGTAATT TCCGCTTCCA GAAGGATTTT  
9301 CTGCGTCCCT TTGAGCATAT CATGAAGAAA AACGCATCGC CGGCAATACG AGATATGGTG  
9361 GTGCGCTGCA TTGCCAGAT GGTAAGTCA CAGGCGCATA ACATCCGTTT CGGCTGGAAG  
9421 AATATCTTTA GCATTTTCCA CCTGGCAGCG GGAGACAACG AAGAGCCAAT TGTGGAGCTG  
9481 GCCTTCCAAA CCACGGGCAA AATCATCGGT GATCTGTACA AGCGTCAGTT CGCCATTATG  
9541 GTGGACTCGT TCCAGGATGC GGTCAAGTGC CTGTCAGAGT TCGCCACCGC CAGATTCCCC  
9601 GATACCAGCA TGGAAGCCAT ACGTCTGGTC CGTACCTGCG CGCAGTGCCT CCACGAGGCA  
9661 CCACAAGTGT TTGCGGAGCA TGCCGGCATG GAGAACGACG CCTCGGTGGC CGAGGAGGAT  
9721 CGAGTCTGGG TCGCGGGCTG GTTTCCGATG CTATTCTCGC TTTCTGCGT GGTCAATCGC  
9781 TGCAAATTGG ATGTGCGTAC TCGCGCCTTA ACCGTGCTTT TTGAGATTGT GAAGACGTAT  
9841 GGTGAGAGCT TCAAGCCCCA TTGGTGAAG GATCTCTTCA ATGTGATCTT CCGTATCTTC  
9901 GACAACATGA AATTGCCGGA GCACGTCACC GAGAAGTCCG AATGGATGAC GACCACATGC  
9961 AACACGCCTT TGTACGCTAT TATTGATGTC TTCACGCAGT ATTTGATGT TCTTGGTCAT  
10021 CTGCTGCTGG AGGAGCTCTT CGCCCAGCTG CATTGGTGTG TTCAGCAGAG TAACGAGCAG  
10081 TTGGCGCGAT CTGGCACCAA TTGCCTGGAG AACCTCGTCA TTTTGAATGG ATTCAAGTTC  
10141 AACGAGTCCA CCTGGGACAA GACGTGCCAG TGCATCCTGG ACATCTTCAA CGCCACTTTG  
10201 CCGCAGGATC TCCTCAGTTG GCGGCCGAAA GCACATTCCA GTAACAATAT ACCCCAGGAG  
10261 CACAACCACT TTGAGGCGCT GCATATCCGC TCGTAGTCC AGCTGGAAC TATACAGACC  
10321 ATGGATAACA TTGTCTTTTT CCCGGCCACG TCGCGCAAGG AGGATGCCGA AACGCTGGCC  
10381 CAGGCGGCGG CAGACTTAAC AGGCGGCAGG AGCGGTTTCG AGTCGCAGCT GCTGGAGTGC  
10441 CAGCGGGAGG AGCAGGGAAT GTACGGCTAT CTGAGAACCC GCCAGCTGCT CACCCTGGCC  
10501 GACTGTCTGA TGCAGTCGCA CCGTTTTGCC AAGCGCTTCA ACGCCGATCA CGACCAACGC  
10561 AGCCTGCTTT GCGGGCGGG ATTCAAGGGA TCTGTAAAC CGAATCTGCT GAAGCAGGAG  
10621 ACCTCGTCGC TGGCCTGCGT CCTGCGCATT TTCTTCAAGA TGTACGGCGA CGAGAATAGA



<pre> 10681 CGCAGCGATT GGCCCGGCAT CGAGCAGGAA CTGGTGCAGG TCTGCAAGGA GGCCTGGGC 10741 TACTATTTGA GTTTGCAGAG CGAGGCACAC CGAGATGCGT GGACATCGCT GCTGCTGCTC 10801 ATCCTGACGC GCCTGCTCAA GATGTCCGAT GCCAGGTTTCG CCACCCACGT TTCCAACCTAC 10861 TACAGCCTGC TGTGCGAGAT GATGTGCTTC GACCTCAAGC CCGAACTGAG AAGTGCCTT 10921 AGGCGTGTGT TCATGCGCAT CGGTCCAGTA TTCAATATAA TGAGCGTTAA ATAAttctag 10981 tcgaccatga agatcaagat cattgccccg ccagagcgca agtactctgt ctgggccctt 11041 cgaaggtaa cctatcccta accctctcct cggctctgat tctacgcgta ccggtcatca 11101 tcaccatcac cattgagttt aaaccgctg atcagcctcg actgtgcctt ctaagatcca 11161 gacatgataa gatacattga tgagtttggg caaaccacaa ctagaatgca gtgaaaaaaaa 11221 tgctttattt gtgaaatttg tgatgctatt gctttatttg taaccatt // </pre>					
<p>pMT-hyg-V5::Sec71<sup>M717L</sup></p>					
LOCUS	pMT_hyg_V5_Sec71_M717L	10266 bp	ds-DNA	circular	28-FEB-2020
DEFINITION	pMT-puro Sequencing Result				
ORGANISM	other sequences; artificial sequences; vectors.				
COMMENT	pMT-vAX2m from 1 to 5573				
COMMENT	pMT-vSOG4m from 1 to 5126				
COMMENT	pMT-mSOGm_v4 from 1 to 5114				
COMMENT	ApEinfo:methylated:1				
FEATURES	Location/Qualifiers				
misc_feature	complement(10177..10196)				
	/label=EBV_rev_primer				
	/ApEinfo_fwdcolor=#ff3600				
	/ApEinfo_revcolor=#ff3600				
	/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}				
	width 5 offset 0				
misc_feature	complement(10134..10151)				
	/label=BGH_rev_primer				
	/ApEinfo_fwdcolor=#ff3600				
	/ApEinfo_revcolor=#ff3600				
	/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}				
	width 5 offset 0				
misc_feature	10094..10111				
	/label=6xHis				
	/ApEinfo_fwdcolor=cyan				

```

/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1} {} 0}
width 5 offset 0
misc_feature 10043..10084
/label=V5
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1} {} 0}
width 5 offset 0
CDS 5014..9969
/label=Sec71
/ApEinfo_fwdcolor=#99ccff
/ApEinfo_revcolor=#cde7f7
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1} {} 0}
width 5 offset 0
misc_feature 9567..9567
/label=T>G silent
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1} {} 0}
width 5 offset 0
misc_feature 10031..10036
/label=ApaI GGGCC^C
/ApEinfo_fwdcolor=#ffffcc
/ApEinfo_revcolor=#ffffcc
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1} {} 0}
width 5 offset 0
primer_bind 7770..7789
/label=sec71-GF6
/ApEinfo_fwdcolor=#ff00bd
/ApEinfo_revcolor=#ff0003
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1} {} 0}
width 5 offset 0
misc_feature 7629..7629
/label=A>G in 40A
/ApEinfo_fwdcolor=cyan
```

```

    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 4927..4932
    /label=KpnI
    /ApEinfo_fwdcolor=#ffffcc
    /ApEinfo_revcolor=#ffffcc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
protein_bind 6733..7305
    /label=Sec7 domain
    /ApEinfo_fwdcolor=#0080ff
    /ApEinfo_revcolor=#0080ff
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 9552..9552
    /label=C>T silent
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind 7250..7273
    /label=Sec71-DRSC01893-F (8A10)
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind 9054..9073
    /label=sec71-GF8
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind 8509..8530
    /label=sec71-GF7
    /ApEinfo_fwdcolor=#ff00bd
```

```
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind    complement(8562..8583)
    /label=Sec71-GR3
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature    6069..6069
    /label=C>T confirmed in 40A 2013
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature    10011..10030
    /label=New Feature
    /ApEinfo_fwdcolor=#ffffcc
    /ApEinfo_revcolor=#ffffcc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature    7557..7557
    /label=T>C in 40A
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature    7668..7668
    /label=A>C in 40A
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind    9662..9679
    /label=Sec71-GF10
    /ApEinfo_fwdcolor=#ff00bd
```

```

    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind    7197..7215
    /label=Sec71-GF11
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind    6797..6820
    /label=Sec71-GF12
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature   7159..7161
    /label=M717(M194 in sec7 domain M>L = BFA-resistant)
    /label=M717L atg>Ctg(BFA-resistant)
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind    6903..6925
    /label=Sec71-GF14
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature   complement(7099..7121)
    /label=Sec71-crRNA7(Protospacer)
    /ApEinfo_fwdcolor=#ccff66
    /ApEinfo_revcolor=#ffff9f
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature   7188..7207
    /label=Sec71-crRNA8 Protospacer
```

```

/ApEinfo_fwdcolor=#ccff66
/ApEinfo_revcolor=#ffffcc
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature 7828..7828
/label=T>C Val>Ala in FRT40A
/ApEinfo_fwdcolor=#ccff66
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
CDS 7890..7890
/label=Sec71-PA
/ApEinfo_fwdcolor=#99ccff
/ApEinfo_revcolor=#cde7f7
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
CDS 5977..5979
/label=Sec71-PA(1)
/ApEinfo_label=Sec71-PA
/ApEinfo_fwdcolor=#99ccff
/ApEinfo_revcolor=#cde7f7
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature 9363..9363
/label=T>C silent
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature 8509..8530
/label=Chang-F1
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind 5568..5587
```

```

        /label=sec71-GF3
        /ApEinfo_fwdcolor=#ff00bd
        /ApEinfo_revcolor=#ff0003
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
misc_feature    7890..7890
        /label=A>C silent
        /ApEinfo_fwdcolor=cyan
        /ApEinfo_revcolor=#00ff00
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
CDS            6069..6069
        /label=Sec71-PA(2)
        /ApEinfo_label=Sec71-PA
        /ApEinfo_fwdcolor=#99ccff
        /ApEinfo_revcolor=#cde7f7
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
CDS            6600..6600
        /label=Sec71-PA(3)
        /ApEinfo_label=Sec71-PA
        /ApEinfo_fwdcolor=#99ccff
        /ApEinfo_revcolor=#cde7f7
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
misc_feature    9447..9447
        /label=G>A silent
        /ApEinfo_fwdcolor=cyan
        /ApEinfo_revcolor=#00ff00
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
misc_feature    7150..7152
        /label=S191
        /ApEinfo_fwdcolor=#66ff66
        /ApEinfo_revcolor=#00ff00
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
```

```
width 5 offset 0
misc_feature 9751..9751
  /label=T>C silent(1)
  /ApEinfo_label=T>C silent
  /ApEinfo_fwdcolor=cyan
  /ApEinfo_revcolor=#00ff00
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind 6550..6569
  /label=Sec71-GF5
  /ApEinfo_fwdcolor=#ff00bd
  /ApEinfo_revcolor=#ff0003
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind complement(7726..7761)
  /label=Sec71-DRSC01893-R
  /ApEinfo_fwdcolor=#ff00bd
  /ApEinfo_revcolor=#ff0003
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 9324..9324
  /label=T>C silent(2)
  /ApEinfo_label=T>C silent
  /ApEinfo_fwdcolor=cyan
  /ApEinfo_revcolor=#00ff00
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind complement(9224..9245)
  /label=Chang-R1
  /ApEinfo_fwdcolor=#fb53d0
  /ApEinfo_revcolor=#fc5a5d
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind 5924..5943
  /label=sec71-GF4
  /ApEinfo_fwdcolor=#ff00bd
```



```

/ApEinfo_revcolor=#ff0003
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature 7039..7041
/ApEinfo_label=E677 (E740 in garz)
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature 6600..6600
/ApEinfo_label=G>A silent(1)
/ApEinfo_label=G>A silent
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature 7147..7149
/ApEinfo_label=F190 F190Y=BFA-hypersensitive
/ApEinfo_label=F713Y(BFA-sensitive)
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature 7719..7719
/ApEinfo_label=A>G 40A
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature 9336..9336
/ApEinfo_label=T>C in 40A(1)
/ApEinfo_label=T>C in 40A
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
```

```
primer_bind    5040..5057
               /label=GF9
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
primer_bind    complement(7375..7398)
               /label=Sec71-GR4
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
primer_bind    8137..8155
               /label=Sec71-GF13
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
misc_feature   9970..9972
               /label=STOP
               /ApEinfo_fwdcolor=#66ccff
               /ApEinfo_revcolor=#00ff00
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
primer_bind    7073..7094
               /label=Sec71-GF15
               /ApEinfo_fwdcolor=#fb53d0
               /ApEinfo_revcolor=#fc5a5d
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
misc_feature   7099..7101
               /label=PAM
               /ApEinfo_fwdcolor=#fc81f0
               /ApEinfo_revcolor=#fc81f0
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
```

```
misc_feature 7208..7210
    /label=PAM(1)
    /ApEinfo_label=PAM
    /ApEinfo_fwdcolor=#fc81f0
    /ApEinfo_revcolor=#fc81f0
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature 4942..4983
    /label=V5(1)
    /ApEinfo_label=V5
    /ApEinfo_fwdcolor=#ffffcc
    /ApEinfo_revcolor=#ffffcc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature 4984..4989
    /label=SpeI
    /ApEinfo_fwdcolor=#ffffcc
    /ApEinfo_revcolor=#ffffcc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature 4990..5013
    /label=Linker GL3
    /ApEinfo_fwdcolor=#ffcc66
    /ApEinfo_revcolor=#ffcc66
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature 4892..4909
    /label=Metallothionein_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature complement(4327..4482)
    /label=lacZ_a
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
```

```
promoter      /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
              width 5 offset 0
              4463..4479
              /label=M13_forward20_primer
              /ApEinfo_fwdcolor=#ccffed
              /ApEinfo_revcolor=#ccffed
              /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
              width 5 offset 0
misc_feature  4448..4470
              /label=M13_pUC_fwd_primer
              /ApEinfo_fwdcolor=#ff3600
              /ApEinfo_revcolor=#ff3600
              /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
              width 5 offset 0
misc_feature  complement(4113..4135)
              /label=pGEX_3_primer
              /ApEinfo_fwdcolor=#ff3600
              /ApEinfo_revcolor=#ff3600
              /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
              width 5 offset 0
promoter      complement(3926..3954)
              /label=AmpR_promoter
              /ApEinfo_fwdcolor=#ccffed
              /ApEinfo_revcolor=#ccffed
              /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
              width 5 offset 0
gene          complement(3024..3884)
              /gene="Ampicillin"
              /label=Ampicillin
              /ApEinfo_fwdcolor=#ffffcc
              /ApEinfo_revcolor=#ffffcc
              /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
              width 5 offset 0
rep_origin    complement(2250..2869)

/translacion="MSIQHFRVALIPFFAAFLPVFAHPETLVKVKDAEDQLGARVGY
```

```
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE

YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLTTIGGPKELTAFLHNMGDHSVTRL

DRWEPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPL

LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
    EIGASLIKHW*"
    /label=pBR322_origin
    /ApEinfo_fwdcolor=pink
    /ApEinfo_revcolor=pink
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
promoter      complement(1912..1941)

/translation="MSIQHFRVALIPFFAAFLPVAHPETLVKVKDAEDQLGARVGY

IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE

YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLTTIGGPKELTAFLHNMGDHSVTRL

DRWEPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPL

LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
    EIGASLIKHW*"
    /label=lac_promoter
    /ApEinfo_fwdcolor=#ccffed
    /ApEinfo_revcolor=#ccffed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature  complement(1876..1898)

/translation="MSIQHFRVALIPFFAAFLPVAHPETLVKVKDAEDQLGARVGY

IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
```

```
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLTTIGGPKELTAFLHNMGDHVTRL
DRWEPELNEAIPNDERDTTMPVAMATTLRKLTLGELLTLASRQQLIDWMEADKVAGPL
LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
    EIGASLIKHW*"
    /label=M13_pUC_rev_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
promoter    complement(1859..1877)

/translation="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGY
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLTTIGGPKELTAFLHNMGDHVTRL
DRWEPELNEAIPNDERDTTMPVAMATTLRKLTLGELLTLASRQQLIDWMEADKVAGPL
LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
    EIGASLIKHW*"
    /label=M13_reverse_primer
    /ApEinfo_fwdcolor=#ccffed
    /ApEinfo_revcolor=#ccffed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    4489..4856
    /label=MT-promoter
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    complement(1350..1708)
```

```

    /label=Copia Promoter?
    /ApEinfo_fwdcolor=#cde7f7
    /ApEinfo_revcolor=#cde7f7
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    4462..4479
    /label=M13-fwd
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=green
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    complement(1859..1879)
    /label=M13-rev
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=green
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
rep_origin    complement(2247..2929)
    /label=ColE1 origin
    /ApEinfo_fwdcolor=gray50
    /ApEinfo_revcolor=gray50
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
CDS            complement(4323..4391)
    /label=LacZ alpha
    /ApEinfo_fwdcolor=#6495ed
    /ApEinfo_revcolor=#6495ed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_binding   complement(1885..1907)
    /label=Lac0
    /ApEinfo_fwdcolor=#6495ed
    /ApEinfo_revcolor=#6495ed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
CDS            complement(3027..3686)
```

```

    /label=AmpR
    /ApEinfo_fwdcolor=yellow
    /ApEinfo_revcolor=yellow
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    4436..4459
    /label=M13F
    /ApEinfo_fwdcolor=#0a00ff
    /ApEinfo_revcolor=#0a00ff
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    4462..4479
    /label=M13F_GW
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    complement(1859..1879)
    /label=M13R_GW
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    3998..4015
    /label=pQE60-F
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    complement(3605..3629)
    /label=Amp-GF
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    3147..3170
```



```

        /label=Amp-GR1
        /ApEinfo_fwdcolor=#ff00bd
        /ApEinfo_revcolor=#ff0003
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
misc_feature    complement(225..1250)
        /label=Hyg
        /ApEinfo_fwdcolor=#ffffcc
        /ApEinfo_revcolor=#00ff00
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
misc_feature    1254..1268
        /label=New Feature(1)
        /ApEinfo_label=New Feature
        /ApEinfo_fwdcolor=cyan
        /ApEinfo_revcolor=#00ff00
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
gene            complement(143..173)
        /gene="puro (variant)"

/translation="MGSCAPFGRALRVVGRASGTGLAGHAPGARSFGHLDVGGDGEAE
PLVEGEVAGRGGLEGGHPGALGRLHSGEHDGAAQTLALVVGRDADGGQEPRGLLGPV
RRQEA FHLLLRGQP GTAQLGHARADLGEHRPRFDALRRGPDRHRGAVVRDPHLADVEP
DAREEEFLQLGDPLDVAVRVDGVARGGVVGERGGEGAYGPGDVVAGGEAHRGLVLGHG
        RSSPCEGSGAWVRGWRRHRSWRPTCRHAS YRIPAARGICCNL*"
        /label=puro (variant)
        /ApEinfo_fwdcolor=#ffffcc
        /ApEinfo_revcolor=#ffffcc
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
misc_feature    95..115
        /label=New Feature(2)
```

```
/ApEinfo_label=New Feature  
/ApEinfo_fwdcolor=cyan  
/ApEinfo_revcolor=#00ff00  
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1} {} 0}  
width 5 offset 0
```

ORIGIN

```
1 ataagctgca ataaacaagt tctagagtcg gtgggcctcg ggggcgggtg cggggtcggc  
61 ggggccgccc cgggtggctt cggtcggagc catggggtcg tgcgctcctt tcggtcgggc  
121 gctgcgggtc gtggggcggg cgtcaggcac cgggcttgcg ggtcatgcac cagttgctgg  
181 ctgaggcggt ctcgaaatca gctcttgttc ggtcggcatc tactctattc ctttgcctc  
241 ggacgagtg cggggcgctg gtttccacta tcggcgagta cttctacaca gccatcggtc  
301 cagacggccg cgcttctgcg ggcgatttgt gtacgcccga cagtcccggc tccggatcgg  
361 acgattgcgt cgcacgacc ctgcgcccga gctgcatcat cgaaattgcc gtcaaccaag  
421 ctctgataga gttggtcaag accaatgcgg agcatatacg cccggagccg cggcgatcct  
481 gcaagctccg gatgcctccg ctggaagtag cgcgtctgct gctccataca agccaaccac  
541 ggcctccaga agaagatggt ggcgacctc gattgggaat ccccgaacat cgcctcgctc  
601 cagtcaatga ccgctgttat gcggccattg tccgtcagga cattggttga gccgaaatcc  
661 gcgtgcacga ggtgccggac ttcggggcag tcctcggccc aaagcatcag ctcatcgaga  
721 gcctgcgcga cggacgcact gacggtgtcg tccatcacag tttgccagtg atacacatgg  
781 ggatcagcaa tcgcgcatat gaaatcacgc catgtagtgt attgaccgat tccttgcggt  
841 ccgaatgggc cgaacccgct cgtctggcta agatcggccg cagcgatcgc atccatggcc  
901 tccgcgaccg gctgcagaac agcgggcagt tcggtttcag gcaggctctg caacgtgaca  
961 ccctgtgcac ggcgggagat gcaataggtc aggctctcgc tgaattcccc aatgtcaagc  
1021 acttccggaa tcgggagcgc ggccgatgca aagtgccgat aaacataacg atctttgtag  
1081 aaaccatcgg cgcagctatt taccgcagg acatatccac gccctctac atcgaagctg  
1141 aaagcacgag attcttcgcc ctccgagagc tgcacaggt cggagacgct gtcgaacttt  
1201 tcgatcagaa acttctcgac agacgtcgcg gtgagttcag gctttttcat ggtggtcgtc  
1261 tccttgtag gggtcagggg cgtgggtcag gggatggtg cggcaccggt cgtggcggcc  
1321 gacctgcagg catgcaagct atcgaattcc tgcagcccgg gggatctggt gtaatttata  
1381 atttatattt ctttcttaa taaataaata aatagtcaag tttatgttg agttttatga  
1441 tttatatttt aagttatttc aactgcaaca ccagcaccac gacctacta cagcaaaaaa  
1501 cgtacaagaa ggaaagaagg aataaaaaga gtggtattct cttacaatat gttttatggc  
1561 ataaaaggtg tggccattca tatcaaatat aaagtagtgt tgtttaacgt tacttttgta  
1621 ggttgaatag tatattccaa cagatgatga ggggttccca atcctaaacc catttgccgt  
1681 tcccagaagc atgaaaccac cacgcaccgg atcctctaga acaacaaca ttgcattcat  
1741 tttatgtttc aggttcaggg ggaggtgtgg gaggtttttt aaagcaagta aaacctctac
```

1801 aaatgtggta tggctgatta tgatcagtcg acctgcaggc atgcaagctt ggcgtaatca  
1861 tggatcatagc tgtttcctgt gtgaaattgt tatccgctca caattccaca caacatacga  
1921 gccggaagca taaagtgtaa agcctggggg gcctaagtag tgagctaaact cacattaatt  
1981 gcgttgcgct cactgcccgc tttccagtcg ggaaacctgt cgtgccagct gcattaatga  
2041 atcggccaac gcgcggggag aggcggtttg cgtattgggc gctcttccgc ttcctcgctc  
2101 actgactcgc tgcgctcggg cgttcggctg cggcgagcgg tatcagctca ctcaaaggcg  
2161 gtaatacggg tatccacaga atcaggggat aacgcaggaa agaacatgtg agcaaaaggc  
2221 cagcaaaagg ccaggaaccg taaaaaggcc gcgttgctgg cgtttttcca taggctccgc  
2281 cccctgacg agcatcacia aaatcgacgc tcaagtcaga ggtggcgaaa cccgacagga  
2341 ctataaagat accaggcgtt tccccctgga agctccctcg tgcgctctcc tgttccgacc  
2401 ctgccgctta ccggatacct gtccgccttt ctcccttcgg gaagcgtggc gctttctcat  
2461 agctcacgct gtaggtatct cagttcgggt taggtcgttc gctccaagct gggctgtgtg  
2521 cacgaacccc ccgttcagcc cgaccgctgc gccttatccg gtaactatcg tcttgagtcc  
2581 aacccggtaa gacacgactt atcgccactg gcagcagcca ctggtaacag gattagcaga  
2641 gcgaggtatg taggcggtgc tacagagttc ttgaagtggg ggcctaacta cggctacact  
2701 agaagaacag tatttggtat ctgcgctctg ctgaagccag ttaccttcgg aaaaagagtt  
2761 ggtagctctt gatccggcaa acaaaccacc gctggtagcg gtggtttttt tgtttgcaag  
2821 cagcagatta cgcgcagaaa aaaaggatct caagaagatc ctttgatctt ttctacgggg  
2881 tctgacgctc agtggaacga aaactcacgt taagggattt tggatcatgag attatcaaaa  
2941 aggatcttca cctagatcct tttaaattaa aatgaagtt taaatcaat ctaaagtata  
3001 tatgagtaaa cttggtctga cagttacca tgcttaatca gtgaggcacc tatctcagcg  
3061 atctgtctat ttcgttcatc catagttgcc tgactccccg tcgtgtagat aactacgata  
3121 cgggagggct taccatctgg cccagtgct gcaatgatac cgcgagacc acgctcaccg  
3181 gctccagatt tatcagcaat aaaccagcca gccggaaggg ccgagcgag aagtggctct  
3241 gcaactttat ccgcctccat ccagtctatt aattgttgcc ggaagctag agtaagtagt  
3301 tcgccagtta atagtttgcg caacgttggt gccattgcta caggcatcgt ggtgtcacgc  
3361 tcgctgtttg gtatggcttc attcagctcc ggttcccaac gatcaaggcg agttacatga  
3421 tccccatgt tgtgcaaaaa agcggtagc tccttcggtc ctccgatcgt tgtcagaagt  
3481 aagttggccg cagtgttatc actcatggtt atggcagcac tgcataattc tcttactgtc  
3541 atgccatccg taagatgctt ttctgtgact ggtgagtact caaccaagtc attctgagaa  
3601 tagtgtatgc ggcgaccgag ttgctcttg cggcgctcaa tacgggataa taccgcgcca  
3661 catagcagaa ctttaaaagt gctcatcatt ggaaaacgtt cttcggggcg aaaactctca  
3721 aggatcttac cgctggttag atccagttcg atgtaacca ctctgtcacc caactgatct  
3781 tcagcatctt ttactttcac cagcgtttct gggtgagcaa aaacaggaag gcaaaatgcc  
3841 gcaaaaaagg gaataagggc gacacggaaa tgttgaatac tcatactctt ctttttcaa  
3901 tattattgaa gcatttatca gggttattgt ctcatgagcg gatacatatt tgaatgtatt

```
3961 tagaaaaata aacaaatagg ggttccgcgc acatttcccc gaaaagtgcc acctgacgtc
4021 taagaaacca ttattatcat gacattaacc tataaaaaata ggcgtatcac gaggcccttt
4081 cgtctcgcgc gtttcggtga tgacggtgaa aacctctgac acatgcagct cccggagacg
4141 gtcacagctt gtctgtaagc ggatgccggg agcagacaag cccgtcaggg cgcgtcagcg
4201 ggtgttggcg ggtgtcgggg ctggcttaac tatgcggcat cagagcagat tgtactgaga
4261 gtgaccata tgcggtgta aataccgcac agatgcgtaa ggagaaaata ccgcatcagg
4321 cgccattcgc cattcaggct gcgcaactgt tgggaagggc gatcggtgcg ggcctcttcg
4381 ctattacgcc agctggcgaa agggggatgt gctgcaaggc gattaagtg ggtaacgcca
4441 gggttttccc agtcacgacg ttgtaaacg acggccagtg aattaattcg ttgcaggaca
4501 ggatgtggtg cccgatgtga ctagctcttt gctgcaggcc gtcctatcct ctggttccga
4561 taagagacc agaactccgg cccccaccg cccaccgcca ccccataca tatgtggtac
4621 gcaagtaaga gtgcctgcgc atgccccatg tgccccacca agagctttgc atcccataca
4681 agtccccaaa gtggagaacc gaaccaattc ttcgcgggca gaacaaaagc ttctgcacac
4741 gtctccactc gaatttggag ccggccggcg tgtgcaaaag aggtgaatcg aacgaaagac
4801 ccgtgtgtaa agccgcgttt ccaaatgta taaaaccgag agcatctggc caatgtgcat
4861 cagttgtggt cagcagcaaa atcaagtga tcatctcagt gcaactaaag gggggatcta
4921 gatcggggta ccgccaccAT GGGTAAGCCT ATCCCTAACC CTCTCCTCGG TCTCGATTCT
4981 ACGACTAGTG GAGGAGGAGG TTCTGGTGGT GGTCACAACA ACTCCACAAA AACCAAGGAA
5041 ATGTTTCATCG TCGTGCTCT AGAAAAGATC CTTGCCGATA AGGACATACG GCGCTCCCAT
5101 CACTCGCAGC TGAAGAAGTC CTGCGATTCTG GCGCTGGAGC AGATTAAGGC GGAGCTAATC
5161 AGTGCCGGCC AGATCGCAGA GGGCAATGAG CTGCCCTGTG CCGCACTCCC GCTGCCAAG
5221 AATGATGCAG CGAGCATCAT AAATGCGGAG ACCTACTTTC TCCCCTTCGA GCTTGCCTGC
5281 AAGAGCCGCT CGCCCAGGAT CGTGGTCACC GCACTGGACT GCCTGCAGAA ACTCATTGCC
5341 TATGGCCATT TGACAGGATC CATTGAGGAC TCGGCCAATC CGGGTCACCT GCTCATCGAC
5401 CGTATCGTTG TGACCATATA TGGCTGCTTC AGTGGTCCCC AGACGGACGA GGCCGTCCAA
5461 CTGAGATAA TAAAGGCTCT GCTCACGGTG GTCACCTCGC AGCATGTGGA AATCCATGAA
5521 TTCACACTGC TGCAAGCTGT GCGCACCTGC TACGACATCT ATTTGTCCAG CAAGAACCTG
5581 GTCAATCAGA CCACAGCACG CGTACGCTC ACCCAAATGT TGAACGTGAT ATTTGCCCGC
5641 ATGGAGAATC AAGTGACGA GCTACCACCT CCCAATTCCA ATCCCACCAA CGGCAGCATC
5701 CACTCGGAGG ATTGCAATGG CTCGGGAGAG GAGTCGCTGC GGGATTCCGA CGAAGTAATT
5761 GCCTCGGAAC TGCTGGCGGA GATCATATCA GCTGCCTACA ATGAGGCGAT GAAGGATCAG
5821 GAATCGGTGC GTGAGCCAGA GCCAACACTT AATGGAAACG ACTACTCCTC GCACTCGGAT
5881 CACGACAGTG TGGAGCTGCA CAGCGAAAAC GATGCGGTTG TAACGGCTAA GTTTACGCAC
5941 ATCCTGCAGA AAGATGCTTT TCTCGTGTTT CCGGCACTGT GCAAGCTATC GATGAAGCCT
6001 TTGCCGGATG GACATCCAGA TCCGAAATCG CACGAGCTGC GTTCCAAGGT GCTGTCATTG
6061 CATCTGCTGC TGCTCATCCT CCAGAATGCC GGGCCCGTCT TCCGCTCCAA CGAGATGTTT
```

6121 ATCATGGCCA TTAAGCAGTA CCTGTGCGTG GCCTTGTCAA ACAACGGAGT CAGTCTGGTG  
6181 CCGGAGGTCT TCGAGCTGTC GCTTTCAATC TTCGTTGCC TACTCTCGAA CTTCAAGGTG  
6241 CATCTTAAGC GGCAGATAGA GGTGTTCTTC AAGGAAATCT TCCTAAACAT TCTTGAGGCG  
6301 AACTCAAGCA GCTTCGAGCA CAAATGGATG GTAATCCAAG CGCTGACACG TATTTGTGCT  
6361 GACGCCCAGT CCGTGGTGG AATCTATGTT AATTACGATT GCGACTTTTC GGCTGCAAAC  
6421 CTTTTTGAGA GACTGGTCAA CGATCTTTCG AAAATTGCC AGGGTCGTCA GGCTCTCGAA  
6481 CTGGGCGCCA ATCCGATGCA AGAGAAATCG ATGCGCATT CCGGCCTGGA GTGTCTTGTC  
6541 TCCATTCTTA AGTGCATGGT AGAGTGGAGT AAGGACTTGT ATGTTAATCC AAACATGCCG  
6601 GTTCCACCTA TGCAAGTCCA ATCGCCGACA AGCACTGAGC AGGATCAGGC GGACACAAC  
6661 ATCCAAACGA TGCACAGTGG TTCCAGTCAT AGTTTGAAC CCAATCAGGA GCAACTACAG  
6721 GATCTTCCCG AGGCATTGGA GGAGCGCAAG ATGCGCAAGG AAGTGATGGA AACAGGCATT  
6781 GAGTTATTCA ATCGTAAGCC TCAGAAAGGA GTGCAATTCC TGCAGGAGAA GCAGTTGCTG  
6841 GGTGCCACAT GCGGGGACAT TGCGCGCTGG CTGCACGAGG ACGAACGACT GGACAAGACA  
6901 GTGATCGGAA ACTACATTGG CGAGAATGAC GACCACTCCA AGGAAGTGAT GTGCGCTTAC  
6961 ATCGATGCCT TTGACTTTCG CCAAATGGAG GTGGTGGCCG CCTTGAGATT TCTTCTCGAG  
7021 GGGTTCCGCC TGCCAGGAGA AGCACAAAA ATCGATCGGC TGATGGAGAA GTTCGCCAGT  
7081 AGATACTGCG AATGCAATCC GAAGAACCAG CTATTCCAAA GCGCAGACAC CGTCTACGTG  
7141 CTGGCATTCA GCATCATTcT GCTGACCACG GATCTTCATT CGCCGAGGT CAAGCACAAAG  
7201 ATGACCAAGG AGCAGTACAT TAAAATGAAC CGCGGCATCA GCGACAGCAA GTCCGATTG  
7261 CCCGAGGAGT ACTTGTGCTC CATCTACGAC GAGATTTCTG AACACGAAAT TAAGATGAAG  
7321 AACAACTCCG GTATGCTTCA ACAGGCGAAA CCCACTGGAA AGCAGGCCTT CATAACGGAG  
7381 AAACGCAGAA AGCTGTTGTG GAACATGGAG ATGGAGGTCA TCTCGCTGAC GGCCACCAAT  
7441 CTAATGCAGT CAGTTTCGCA CGTCAAGTCA CCCTTCACCT CAGCGAAACA CTTGGAGCAT  
7501 GTCCGGCCCA TGTTCAAAT GGCTTGGACA CCATTTCTGG CCGCTTCTC TGTGGGTCTC  
7561 CAGGACTGCG ACGATCCTGA GATTGCTACA CTCTGCTTG ATGGTATACG TTGTGCTATT  
7621 CGAATCGCAT GCATCTTCCA CATGTCCCTG GAGCGAGATG CCTATGTACA AGCCCTGGCC  
7681 AGGTTTACTC TCCTGAATGC TAACTCGCCC ATCAACGAAA TGAAGGCCAA GAATATCGAT  
7741 ACCATCAAGA CGCTTATAAT GGTAGCCAC ACGGATGGCA ATTATCTGG CAGCAGCTGG  
7801 CTGGATATAG TGAAGTGCAT TAGCCAGTTG GAGCTGGCCC AACTGATCG CACTGGGGTG  
7861 CGGCCCCAGT TTCTTTCTGG AGCGCAGACA ACGCTCAAG ACTCGTTAA TCCAGCGTG  
7921 AAAGAACACA TCGGCGAGAC GAGCAGCCAG AGCGTGGTGG TCGCAGTCGA TCGTATTTTC  
7981 ACCGGCTCAA TGCGACTGGA TGGCGATGCT ATCGTGGACT TCGTGAAGGC CCTGTGCCAG  
8041 GTGTCTGTGG ATGAGCTTCA GCAGCAGCAA CCGAGGATGT TCTCCTTGCA AAAGATAGTG  
8101 GAAATTAGTT ACTACAACAT GGAGCGTATT CGTCTGCAGT GGTCACGCAT TTGGCAAGTT  
8161 TTGGGTGAGC ACTTTAACGC GGTCGGATGC AATAGCAACG AGGAGATCTC ATTTTTCGCC  
8221 CTGGACTCAC TGCGTCAGTT GTCGATGAAG TTCATGGAGA AGGGCGAGTT CAGTAATTC

8281 CGCTTCCAGA AGGATTTCTT GCGTCCCTTT GAGCATATCA TGAAGAAAAA CGCATCGCCG  
8341 GCAATACGAG ATATGGTGGT GCGCTGCATT GCCCAGATGG TAAACTCACA GGCGCATAAC  
8401 ATCCGTTCCG GCTGGAAGAA TATCTTTAGC ATTTTCCACC TGGCAGCGGG AGACAACGAA  
8461 GAGCCAATTG TGGAGCTGGC CTTCCAAACC ACGGGCAAAA TCATCGGTGA TCTGTACAAG  
8521 CGTCAGTTCG CCATTATGGT GGA CTGTTT CAGGATGCGG TCAAGTGCCT GTCAGAGTTC  
8581 GCCACCGCCA GATTCCCCGA TACCAGCATG GAAGCCATAC GTCTGGTCCG TACCTGCGCG  
8641 CAGTGCCTCC ACGAGGCACC ACAACTGTTT GCGGAGCATG CCGGCATGGA GAACGACGCC  
8701 TCGGTGGCCG AGGAGGATCG AGTCTGGGTG CGCGGCTGGT TTCCGATGCT ATTCTCGCTT  
8761 TCCTGCGTGG TCAATCGCTG CAAATTGGAT GTGCGTACTC GCGCCTTAAC CGTGCTTTTT  
8821 GAGATTGTGA AGACGTATGG TGAGAGCTTC AAGCCCCATT GGTGGAAGGA TCTCTTCAAT  
8881 GTGATCTTCC GTATCTTCGA CAACATGAAA TTGCCGGAGC ACGTCACCGA GAAGTCCGAA  
8941 TGGATGACGA CCACATGCAA CCACGCCTTG TACGCTATTA TTGATGTCTT CACGCAGTAT  
9001 TTCGATGTTT TTGGTCATCT GCTGCTGGAG GAGCTCTTCG CCCAGCTGCA TTGGTGTGTT  
9061 CAGCAGAGTA ACGAGCAGTT GGCGCGATCT GGCACCAATT GCCTGGAGAA CCTCGTCATT  
9121 TCGAATGGAT TCAAGTTCAA CGAGTCCACC TGGGACAAGA CGTGCCAGTG CATCCTGGAC  
9181 ATCTTCAACG CCACTTTGCC GCAGGATCTC CTCAGTTGGC GGCCGAAAGC ACATTCCAGT  
9241 AACAAATATAC CCCAGGAGCA CAACCACTTT GAGGCGCTGC ATATCCGCTG CGTAGTCCAG  
9301 CTGGAAGTGA TACAGACCAT GGATAACATT GTCTTTTTCC CGGCCACGTC GCGCAAGGAG  
9361 GATGCCGAAA CGCTGGCCCA GGCGGCGGCA GACTTAACAG GCGGCAGGAG CGGTTCGCAG  
9421 TCGCAGCTGC TGGAGTGCCA GCGGGAGGAG CAGGGAATGT ACGGCTATCT GAGAACCCGC  
9481 CAGCTGCTCA CCCTGGCCGA CTGTCTGATG CAGTCGCACC GTTTTGCCAA GCGCTTCAAC  
9541 GCCGATCACG ACCAACGCAG CCTGCTTTGG CGGGCGGGAT TCAAGGGATC TGTTAAACCG  
9601 AATCTGCTGA AGCAGGAGAC CTCGTCGCTG GCCTGCGTCC TGCGCATTTT CTTCAAGATG  
9661 TACGGCGACG AGAATAGACG CAGCGATTGG CCCGGCATCG AGCAGGAACT GGTGCAGGTC  
9721 TGCAAGGAGG CACTGGGCTA CTATTTGAGT TTGCAGAGCG AGGCACACCG AGATGCGTGG  
9781 ACATCGCTGC TGCTGCTCAT CCTGACGCGC CTGCTCAAGA TGTCCGATGC CAGGTTCCGC  
9841 ACCCACGTTT CCAACTACTA CAGCCTGCTG TGCGAGATGA TGTGCTTCGA CCTCAAGCCC  
9901 GAACTGAGAA GTGTCCTTAG GCGTGTGTTT ATGCGCATCG GTCCAGTATT CAATATAATG  
9961 AGCGTTAAAT AAttctagtc gaccatgaag atcaagatca ttgccccgcc agagcgcgaag  
10021 tactctgtct gggcccttcg aaGGTAAGCC TATCCCTAAC CCTCTCCTCG GTCTCGATTC  
10081 TACGcgtacc ggtCATCATC ACCATCACCA TTGAgtttta acccgctgat cagcctcgac  
10141 tgtgccttct aagatccaga catgataaga tacattgatg agtttggaca aaccacaact  
10201 agaatgcagt gaaaaaatg ctttatttgt gaaatttgtg atgctattgc tttatttghta  
10261 accatt

//

pMT-hyg-V5-iRFP713-Sec71<sup>M717L</sup>

```
LOCUS      pMT_hyg_V5_RFP713-Sec71_M717L      11268 bp ds-DNA      circular
28-FEB-2020
DEFINITION pMT-puro Sequencing Result
ORGANISM   other sequences; artificial sequences; vectors.
COMMENT    pMT-mAX2m-blast from 1 to 5500
COMMENT    pMT-mAX2m-neo from 1 to 5813
COMMENT    pMT-mAPEX2m from 1 to 5561
COMMENT    pMT-mSOG1m from 1 to 5078
COMMENT    pMT-mSOG4m from 1 to 5114
COMMENT    ApEinfo:methylated:0
FEATURES   Location/Qualifiers
    misc_feature   4892..4909
                    /label=Metallothionein_primer
                    /ApEinfo_fwdcolor=#ff3600
                    /ApEinfo_revcolor=#ff3600
                    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                    width 5 offset 0
    misc_feature   4927..4932
                    /label=KpnI
                    /ApEinfo_fwdcolor=#ffffcc
                    /ApEinfo_revcolor=#ffffcc
                    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                    width 5 offset 0
    misc_feature   complement(225..1250)
                    /label=Hyg
                    /ApEinfo_fwdcolor=#ffffcc
                    /ApEinfo_revcolor=#00ff00
                    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                    width 5 offset 0
    gene          complement(143..173)
                    /gene="puro (variant)"

/translacion="MGSCAPFGRALRVVGRASGTGLAGHAPGARSFGHLDVGGDGEAE
PLVEGEVAGRGGLEGGHPGALGRLHSGEHDGAAQTLALVVGRDADGGQEPRGLLGPV
```

```
RRQEAFHLLL RGQPGTAQLGHARADLGEHRPRFDALRRGPDRHRGAVVRDPHLADVEP

DAREEEFLQLGDPLDVAVRVDGVARGGVVGERGGEGAYGPGDVVAGGEAHRGLVLGHG
    RSPCEGSGAWVRGWRRHSWRPTCRHASYRIPAARGICCNL*"
    /label=puro (variant)
    /ApEinfo_fwdcolor=#ffffcc
    /ApEinfo_revcolor=#ffffcc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    95..115
    /label=New Feature
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
promoter        4463..4479
    /label=M13_forward20_primer
    /ApEinfo_fwdcolor=#ccffed
    /ApEinfo_revcolor=#ccffed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    4448..4470
    /label=M13_pUC_fwd_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
promoter        complement(3926..3954)
    /label=AmpR_promoter
    /ApEinfo_fwdcolor=#ccffed
    /ApEinfo_revcolor=#ccffed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
rep_origin      complement(2250..2869)

/translacion="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGY
```



```
I ELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLTTIGGPKELTAFLHNMGDHSVTRL
DRWEPELNEAIPNDERDTMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPL

LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
    EIGASLIKHW*"
    /label=pBR322_origin
    /ApEinfo_fwdcolor=pink
    /ApEinfo_revcolor=pink
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
CDS      6016..10971
    /label=Sec71
    /ApEinfo_fwdcolor=#99ccff
    /ApEinfo_revcolor=#cde7f7
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    complement(1876..1898)

/translation="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGY
I ELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLTTIGGPKELTAFLHNMGDHSVTRL
DRWEPELNEAIPNDERDTMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPL

LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
    EIGASLIKHW*"
    /label=M13_pUC_rev_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
```

```
width 5 offset 0
misc_feature complement(1350..1708)
  /label=Copia Promoter?
  /ApEinfo_fwdcolor=#cde7f7
  /ApEinfo_revcolor=#cde7f7
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 10569..10569
  /label=T>G silent
  /ApEinfo_fwdcolor=cyan
  /ApEinfo_revcolor=#00ff00
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature complement(4327..4482)
  /label=lacZ_a
  /ApEinfo_fwdcolor=#ff3600
  /ApEinfo_revcolor=#ff3600
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature complement(4113..4135)
  /label=pGEX_3_primer
  /ApEinfo_fwdcolor=#ff3600
  /ApEinfo_revcolor=#ff3600
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind 8772..8791
  /label=sec71-GF6
  /ApEinfo_fwdcolor=#ff00bd
  /ApEinfo_revcolor=#ff0003
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
gene complement(3024..3884)
  /gene="Ampicillin"
  /label=Ampicillin
  /ApEinfo_fwdcolor=#ffffcc
  /ApEinfo_revcolor=#ffffcc
```

```

                                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                                width 5 offset 0
promoter      complement(1912..1941)

/translacion="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGY
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHVTRL
DRWEPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPL
LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
                                EIGASLIKHW*"
                                /label=lac_promoter
                                /ApEinfo_fwdcolor=#ccffed
                                /ApEinfo_revcolor=#ccffed
                                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                                width 5 offset 0
misc_feature  8631..8631
                                /label=A>G in 40A
                                /ApEinfo_fwdcolor=cyan
                                /ApEinfo_revcolor=#00ff00
                                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                                width 5 offset 0
promoter      complement(1859..1877)

/translacion="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGY
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHVTRL
DRWEPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPL
LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
```

```
EIGASLIKHW*"
  /label=M13_reverse_primer
  /ApEinfo_fwdcolor=#ccffed
  /ApEinfo_revcolor=#ccffed
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 4489..4856
  /label=MT-promoter
  /ApEinfo_fwdcolor=cyan
  /ApEinfo_revcolor=#00ff00
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
protein_bind 7735..8307
  /label=Sec7 domain
  /ApEinfo_fwdcolor=#0080ff
  /ApEinfo_revcolor=#0080ff
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
CDS complement(4323..4391)
  /label=LacZ alpha
  /ApEinfo_fwdcolor=#6495ed
  /ApEinfo_revcolor=#6495ed
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind 3998..4015
  /label=pQE60-F
  /ApEinfo_fwdcolor=#ff00bd
  /ApEinfo_revcolor=#ff0003
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 10554..10554
  /label=C>T silent
  /ApEinfo_fwdcolor=cyan
  /ApEinfo_revcolor=#00ff00
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
```

```
primer_bind    complement(1859..1879)
               /label=M13R_GW
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
primer_bind    complement(1859..1879)
               /label=M13-rev
               /ApEinfo_fwdcolor=cyan
               /ApEinfo_revcolor=green
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
primer_bind    8252..8275
               /label=Sec71-DRSC01893-F (8A10)
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
primer_bind    4462..4479
               /label=M13-fwd
               /ApEinfo_fwdcolor=cyan
               /ApEinfo_revcolor=green
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
primer_bind    4436..4459
               /label=M13F
               /ApEinfo_fwdcolor=#0a00ff
               /ApEinfo_revcolor=#0a00ff
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
primer_bind    10056..10075
               /label=sec71-GF8
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
```

```
primer_bind    4832..4849
               /label=SEQ-MT-F2
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
rep_origin     complement(2247..2929)
               /label=ColE1 origin
               /ApEinfo_fwdcolor=gray50
               /ApEinfo_revcolor=gray50
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
primer_bind    9511..9532
               /label=sec71-GF7
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
CDS            complement(3027..3686)
               /label=AmpR
               /ApEinfo_fwdcolor=yellow
               /ApEinfo_revcolor=yellow
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
misc_binding   complement(1885..1907)
               /label=Lac0
               /ApEinfo_fwdcolor=#6495ed
               /ApEinfo_revcolor=#6495ed
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
primer_bind    complement(9564..9585)
               /label=Sec71-GR3
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
```

```
primer_bind    complement(3605..3629)
               /label=Amp-GF
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
primer_bind    3147..3170
               /label=Amp-GR1
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
misc_feature   7071..7071
               /label=C>T confirmed in 40A 2013
               /ApEinfo_fwdcolor=cyan
               /ApEinfo_revcolor=#00ff00
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
primer_bind    4462..4479
               /label=M13F_GW
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
primer_bind    1737..1759
               /label=Seq-EB-R
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
misc_feature   8559..8559
               /label=T>C in 40A
               /ApEinfo_fwdcolor=cyan
               /ApEinfo_revcolor=#00ff00
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
```

```
misc_feature 1254..1268
  /label=New Feature(1)
  /ApEinfo_label=New Feature
  /ApEinfo_fwdcolor=cyan
  /ApEinfo_revcolor=#00ff00
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0

misc_feature 8670..8670
  /label=A>C in 40A
  /ApEinfo_fwdcolor=cyan
  /ApEinfo_revcolor=#00ff00
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0

misc_feature complement(11179..11198)
  /label=EBV_rev_primer
  /ApEinfo_fwdcolor=#ff3600
  /ApEinfo_revcolor=#ff3600
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0

misc_feature complement(11136..11153)
  /label=BGH_rev_primer
  /ApEinfo_fwdcolor=#ff3600
  /ApEinfo_revcolor=#ff3600
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0

misc_feature 11096..11113
  /label=6xHis
  /ApEinfo_fwdcolor=cyan
  /ApEinfo_revcolor=#00ff00
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0

misc_feature 11045..11086
  /label=V5
  /ApEinfo_fwdcolor=cyan
  /ApEinfo_revcolor=#00ff00
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
```



```
width 5 offset 0
misc_feature 11013..11032
  /label=New Feature(2)
  /ApEinfo_label=New Feature
  /ApEinfo_fwdcolor=#ffffcc
  /ApEinfo_revcolor=#ffffcc
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 11033..11038
  /label=ApaI GGGCC^C
  /ApEinfo_fwdcolor=#ffffcc
  /ApEinfo_revcolor=#ffffcc
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 5995..6015
  /label=Linker GL3
  /ApEinfo_fwdcolor=#ffcc66
  /ApEinfo_revcolor=#00ff00
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 5992..5994
  /label=Linker GL3(1)
  /ApEinfo_label=Linker GL3
  /ApEinfo_fwdcolor=#ffcc66
  /ApEinfo_revcolor=#00ff00
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 8152..8154
  /label=S191
  /ApEinfo_fwdcolor=#66ff66
  /ApEinfo_revcolor=#00ff00
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
CDS 5035..5982
  /codon_start=1
  /product="phytochrome-based near-infrared fluorescent
```

```
protein, originally termed iRFP (Filonov et al., 2011)"
/note="name was changed to iRFP713 (Shcherbakova and
Verkhusha, 2013)"

/translation="MAEGSVARQPDLLTCDEPIHIPGAIQPHGLLLALAADMIVAG
S
DNLPELTGLAIGALIGRSAADVFDSETHNRLTIALAEPGAAVGAPITVGFTMRKDAGF
I
GSWHRHDQLIFLELEPPQRDVAEPQAFFRRTNSAIRRLQAAETLESACAAAAQEVKRI
T
GFDRVMIYRFASDFSGEVIAEDRCAEVESKLGHPASTVPAQARRLYTINPVRIIPD
I
NYRPVPVTPDLNPVTGRPIDLSFAILRSVSPVHLEFMRNIGMGTMSISILRGERLWG
L IVCHHRTPYYVDLDGRQACELVAQVLAWQIGVMEE"
/label=iRFP713
/ApEinfo_fwdcolor=#9a69fe
/ApEinfo_revcolor=#cde7f7
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature 10326..10326
/label=T>C silent
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature 4942..4983
/label=V5(1)
/ApEinfo_label=V5
/ApEinfo_fwdcolor=#ffffcc
/ApEinfo_revcolor=#ffffcc
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
```

```
primer_bind    complement(10226..10247)
               /label=Chang-R1
               /ApEinfo_fwdcolor=#fb53d0
               /ApEinfo_revcolor=#fc5a5d
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
misc_feature    4984..4989
               /label=SpeI
               /ApEinfo_fwdcolor=#ffffcc
               /ApEinfo_revcolor=#ffffcc
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
primer_bind    6926..6945
               /label=sec71-GF4
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
misc_feature    4990..5013
               /label=Linker GL3(2)
               /ApEinfo_label=Linker GL3
               /ApEinfo_fwdcolor=#ffcc66
               /ApEinfo_revcolor=#00ff00
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
misc_feature    8041..8043
               /label=E677 (E740 in garz)
               /ApEinfo_fwdcolor=cyan
               /ApEinfo_revcolor=#00ff00
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
               width 5 offset 0
regulatory     5029..5038
               /regulatory_class="other"
               /note="vertebrate consensus sequence for strong
initiation
               of translation (Kozak, 1987)"
```

```
initiation      /label=vertebrate consensus sequence for strong
of translation (Kozak, 1987)
/ApEinfo_fwdcolor=pink
/ApEinfo_revcolor=pink
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature    7602..7602
/label=G>A silent
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind     8199..8217
/label=Sec71-GF11
/ApEinfo_fwdcolor=#ff00bd
/ApEinfo_revcolor=#ff0003
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind     7799..7822
/label=Sec71-GF12
/ApEinfo_fwdcolor=#ff00bd
/ApEinfo_revcolor=#ff0003
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature    8161..8163
/label=M717(M194 in sec7 domain M>L = BFA-resistant)
/label=M717L atg>Ctg(BFA-resistant)
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind     7905..7927
/label=Sec71-GF14
/ApEinfo_fwdcolor=#ff00bd
/ApEinfo_revcolor=#ff0003
```

```
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    complement(8101..8123)
    /label=Sec71-crRNA7(Protospacer)
    /ApEinfo_fwdcolor=#ccff66
    /ApEinfo_revcolor=#ffff9f
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    8190..8209
    /label=Sec71-crRNA8 Protospacer
    /ApEinfo_fwdcolor=#ccff66
    /ApEinfo_revcolor=#ffffcc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    8830..8830
    /label=T>C Val>Ala in FRT40A
    /ApEinfo_fwdcolor=#ccff66
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
CDS            8892..8892
    /label=Sec71-PA
    /ApEinfo_fwdcolor=#99ccff
    /ApEinfo_revcolor=#cde7f7
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
CDS            6979..6981
    /label=Sec71-PA(1)
    /ApEinfo_label=Sec71-PA
    /ApEinfo_fwdcolor=#99ccff
    /ApEinfo_revcolor=#cde7f7
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    10365..10365
    /label=T>C silent(1)
    /ApEinfo_label=T>C silent
```

```

/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 9511..9532
/label=Chang-F1
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind 6570..6589
/label=sec71-GF3
/ApEinfo_fwdcolor=#ff00bd
/ApEinfo_revcolor=#ff0003
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 8892..8892
/label=A>C silent
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
CDS 7071..7071
/label=Sec71-PA(2)
/ApEinfo_label=Sec71-PA
/ApEinfo_fwdcolor=#99ccff
/ApEinfo_revcolor=#cde7f7
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
CDS 7602..7602
/label=Sec71-PA(3)
/ApEinfo_label=Sec71-PA
/ApEinfo_fwdcolor=#99ccff
/ApEinfo_revcolor=#cde7f7
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
```

```
misc_feature    10449..10449
                /label=G>A silent(1)
                /ApEinfo_label=G>A silent
                /ApEinfo_fwdcolor=cyan
                /ApEinfo_revcolor=#00ff00
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
misc_feature    10753..10753
                /label=T>C silent(2)
                /ApEinfo_label=T>C silent
                /ApEinfo_fwdcolor=cyan
                /ApEinfo_revcolor=#00ff00
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
primer_bind     7552..7571
                /label=Sec71-GF5
                /ApEinfo_fwdcolor=#ff00bd
                /ApEinfo_revcolor=#ff0003
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
primer_bind     complement(8728..8763)
                /label=Sec71-DRSC01893-R
                /ApEinfo_fwdcolor=#ff00bd
                /ApEinfo_revcolor=#ff0003
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
misc_feature    8149..8151
                /label=F190 F190Y=BFA-hypersensitive
                /label=F713Y(BFA-sensitive)
                /ApEinfo_fwdcolor=cyan
                /ApEinfo_revcolor=#00ff00
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
misc_feature    8721..8721
                /label=A>G 40A
                /ApEinfo_fwdcolor=cyan
```

```

/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 10338..10338
/label=T>C in 40A(1)
/ApEinfo_label=T>C in 40A
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind 6042..6059
/label=GF9
/ApEinfo_fwdcolor=#ff00bd
/ApEinfo_revcolor=#ff0003
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind complement(8377..8400)
/label=Sec71-GR4
/ApEinfo_fwdcolor=#ff00bd
/ApEinfo_revcolor=#ff0003
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind 9139..9157
/label=Sec71-GF13
/ApEinfo_fwdcolor=#ff00bd
/ApEinfo_revcolor=#ff0003
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 10972..10974
/label=STOP
/ApEinfo_fwdcolor=#66ccff
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind 8075..8096
/label=Sec71-GF15
```



```
    /ApEinfo_fwdcolor=#fb53d0
    /ApEinfo_revcolor=#fc5a5d
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    8101..8103
    /label=PAM
    /ApEinfo_fwdcolor=#fc81f0
    /ApEinfo_revcolor=#fc81f0
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    8210..8212
    /label=PAM(1)
    /ApEinfo_label=PAM
    /ApEinfo_fwdcolor=#fc81f0
    /ApEinfo_revcolor=#fc81f0
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    10664..10681
    /label=Sec71-GF10
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
```

ORIGIN

```
1 ataagctgca ataaacaagt tctagagtcg gtgggcctcg ggggcgggtg cggggtcggc
61 ggggccgcc cgggtggctt cggtcggagc catggggtcg tgcgctcctt tcggtcgggc
121 gctgcgggtc gtggggcggg cgtcaggcac cgggcttgcg ggtcatgcac cagttgctgg
181 ctgaggcggtt ctgaaatca gctcttgttc ggtcggcatc tactctattc ctttgcctc
241 ggacgagtgc tggggcgtcg gtttccacta tcggcgagta cttctacaca gccatcggtc
301 cagacggccg cgcttctgcg ggcgatttgt gtacgcccga cagtcccggc tccggatcgg
361 acgattgcgt cgcacgacc ctgcgccaa gctgcatcat cgaaattgcc gtcaaccaag
421 ctctgataga gttggtcaag accaatgcgg agcatatacg cccggagccg cggcgatcct
481 gcaagctccg gatgcctccg ctggaagtag cgcgtctgct gctccataca agccaaccac
541 ggcctccaga agaagatggt ggcgacctcg tattgggaat ccccgaacat cgcctcgctc
601 cagtcaatga ccgctgttat gcggccattg tccgtcagga cattgttggg gccgaatcc
661 gcgtgcacga ggtgccggac ttcggggcag tcctcggccc aaagcatcag ctcatcgaga
```

721 gcctgcgcga cggacgcact gacggtgtcg tccatcacag tttgccagtg atacacatgg  
781 ggatcagcaa tcgcgcatat gaaatcacgc catgtagtgt attgaccgat tccttgcggt  
841 ccgaatgggc cgaacccgct cgtctggcta agatcggccg cagcgatcgc atccatggcc  
901 tccgcgaccg gctgcagaac agcgggcagt tcggtttcag gcaggctctg caacgtgaca  
961 ccctgtgcac ggcgggagat gcaataggtc aggctctcgc tgaattcccc aatgtcaagc  
1021 acttccggaa tcgggagcgc ggccgatgca aagtgccgat aaacataacg atctttgtag  
1081 aaaccatcgg cgcagctatt taccgcagg acatatccac gccctctac atcgaagctg  
1141 aaagcacgag attcttcgcc ctccgagagc tgcatacagg cggagacgct gtcgaacttt  
1201 tcgatcagaa acttctcgac agacgtcgcg gtgagttcag gctttttcat ggtggtcgtc  
1261 tccttgtgag gggtcagggg cgtgggtcag gggatggtgg cggcaccggt cgtggcggcc  
1321 gacctgcagg catgcaagct atcgaattcc tgcagcccgg gggatctgtt gtaatttata  
1381 atttatattt cttttcttaa taaataaata aatagtcaag tttatgttg agttttatga  
1441 tttatatttt aagttatttc aactgcaaca ccagcaccac gacctacta cagcaaaaaa  
1501 cgtacaagaa ggaagaagg aataaaaaga gtggtattct cttacaatat gttttatggc  
1561 ataaaagggt tggccattca tatcaaatat aaagtagtgt tgtttaacgt tacttttcta  
1621 ggttgaatag tatattccaa cagatgatga ggggttccca atcctaaacc catttgccgt  
1681 tcccagaagc atgaaaccac cacgcaccgg atcctctaga acaacaaca ttgcattcat  
1741 tttatgtttc aggttcaggg ggaggtgtgg gaggtttttt aaagcaagta aaacctctac  
1801 aatgttggtg tggctgatta tgatcagtcg acctgcaggc atgcaagctt ggcgtaatca  
1861 tggcatagc tgtttcctgt gtgaaattgt tatccgctca caattccaca caacatacga  
1921 gccggaagca taaagtgtaa agcctggggg gcctaagtag tgagctaact cacattaatt  
1981 gcgttgcgct cactgcccgc tttccagtcg ggaaacctgt cgtgccagct gcattaatga  
2041 atcggccaac gcgcggggag aggcggtttg cgtattgggc gctcttcgc ttcctcgctc  
2101 actgactcgc tgcgctcggc cgttcggctg cggcgagcgg tatcagctca ctcaaaggcg  
2161 gtaatacggc tatccacaga atcaggggat aacgcaggaa agaacatgtg agcaaaaggc  
2221 cagcaaaagg ccaggaaccg taaaaggcc gcgttgctgg cgtttttcca taggctccgc  
2281 ccccctgacg agcatcaca aaatcgacgc tcaagtcaga ggtggcgaaa cccgacagga  
2341 ctataaagat accaggcgtt tccccctgga agctccctcg tgcgctctcc tgttccgacc  
2401 ctgccgctta ccggatacct gtccgccttt ctcccttcgg gaagcgtggc gctttctcat  
2461 agctcacgct gtaggtatct cagttcgggt taggtcgttc gctccaagct gggctgtgtg  
2521 cacgaacccc ccgttcagcc cgaccgctgc gccttatccg gtaactatcg tcttgagtcc  
2581 aacccggtaa gacacgactt atcgccactg gcagcagcca ctggtaacag gattagcaga  
2641 gcgaggtatg taggcggtgc tacagagttc ttgaagtgtt ggcctaacta cggctacact  
2701 agaagaacag tatttggtat ctgcgctctg ctgaagccag ttaccttcgg aaaaagagtt  
2761 ggtagctctt gatccggcaa acaaacacc gctggtagcg gtgggttttt tgtttgcaag  
2821 cagcagatta cgcgcagaaa aaaaggatct caagaagatc ctttgcctt ttctacgggg

```
2881 tctgacgctc agtggaacga aaactcacgt taagggattt tggatcatgag attatcaaaa
2941 aggatcttca cctagatcct tttaaattaa aatgaagtt ttaaatcaat ctaaagtata
3001 tatgagtaaa cttggtctga cagttaccaa tgcttaatca gtgaggcacc tatctcagcg
3061 atctgtctat ttcgttcate catagttgcc tgactccccg tcgtgtagat aactacgata
3121 cgggagggct taccatctgg cccagtgct gcaatgatac cgcgagacc acgctcaccg
3181 gctccagatt tatcagcaat aaaccagcca gccggaaggg ccgagcgag aagtggctct
3241 gcaactttat ccgctccat ccagtctatt aattggtgcc ggaagctag agtaagtagt
3301 tcgccagtta atagtttgcg caacgttgtt gccattgcta caggcatcgt ggtgtcacgc
3361 tcgtcgtttg gtatggcttc attcagctcc ggttcccaac gatcaaggcg agttacatga
3421 tccccatgt tgtgcaaaaa agcggtagc tccttcggtc ctccgatcgt tgtcagaagt
3481 aagttggccg cagtgttate actcatggtt atggcagcac tgcataattc tcttactgtc
3541 atgccatccg taagatgctt ttctgtgact ggtgagtact caaccaagtc attctgagaa
3601 tagtgtatgc ggcgaccgag ttgctcttgc ccggcgtcaa tacgggataa taccgcgcca
3661 catagcagaa ctttaaaagt gctcatcatt ggaaaacgtt cttcggggcg aaaactctca
3721 aggatcttac cgctgttgag atccagttcg atgtaacca ctctgtcacc caactgatct
3781 tcagcatctt ttactttcac cagcgtttct gggtgagcaa aaacaggaag gcaaaatgcc
3841 gcaaaaaagg gaataagggc gacacggaaa tgttgaatac tcatactctt ctttttcaa
3901 tattattgaa gcatttatca gggttattgt ctcatgagcg gatacatatt tgaatgtatt
3961 tagaaaaata aacaaatagg ggttccgcgc acatttccc gaaaagtgcc acctgacgtc
4021 taagaaacca ttattatcat gacattaacc tataaaaaata ggcgtatcac gaggcccttt
4081 cgtctcgcgc gtttcggtga tgacggtgaa aacctctgac acatgcagct cccggagacg
4141 gtcacagctt gtctgtaagc ggatgccggg agcagacaag cccgtcaggg cgcgtcagcg
4201 ggtgttggcg ggtgtcgggg ctggcttaac tatgcggcat cagagcagat tgtactgaga
4261 gtgcaccata tgcggtgtga aataccgcac agatgcgtaa ggagaaaata ccgcatcagg
4321 cgccattcgc cattcaggct gcgcaactgt tgggaagggc gatcgggtcg ggcctcttcg
4381 ctattacgcc agctggcgaa agggggatgt gctgcaaggc gattaagttag ggtaacgcca
4441 gggttttccc agtcacgacg ttgtaaaacg acggccagtg aattaattcg ttgcaggaca
4501 ggatgtgggtg cccgatgtga ctagctcttt gctgcaggcc gtcctatcct ctggttccga
4561 taagagacc agaactccgg cccccaccg cccaccgcca ccccataca tatgtggtac
4621 gcaagtaaga gtgcctgcgc atgccccatg tgccccacca agagctttgc atccataca
4681 agtccccaaa gtggagaacc gaaccaattc ttcgcgggca gaacaaaagc ttctgcacac
4741 gtctccactc gaatttggag ccggccggcg tgtgcaaaag aggtgaatcg aacgaaagac
4801 ccgtgtgtaa agccgcgttt ccaaaatgta taaaaccgag agcatctggc caatgtgcat
4861 cagttgtggc cagcagcaaa atcaagttaa tcatctcagt gcaactaaag gggggatcta
4921 gatcggggta ccgccaccAT GGGTAAGCCT ATCCCTAACC CTCTCCTCGG TCTCGATTCT
4981 ACGACTAGTG GAGGAGGAGG TTCTGGTGGT GGTGCGGCCG CATCTGCCGC CACCATGGCT
```

5041 GAAGGATCCG TCGCCAGGCA GCCTGACCTC TTGACCTGCG ACGATGAGCC GATCCATATC  
5101 CCCGGTGCCA TCCAACCGCA TGGACTGCTG CTCGCCCTCG CCGCCGACAT GACGATCGTT  
5161 GCCGGCAGCG ACAACCTTCC CGAACTCACC GGACTGGCGA TCGGCGCCCT GATCGGCCGC  
5221 TCTGCGGCCG ATGTCTTCGA CTCGGAGACG CACAACCGTC TGACGATCGC CTTGGCCGAG  
5281 CCCGGGGCGG CCGTCGGAGC ACCGATCACT GTCGGCTTCA CGATGCGAAA GGACGCAGGC  
5341 TTCATCGGCT CCTGGCATCG CCATGATCAG CTCATCTTCC TCGAGCTCGA GCCTCCCCAG  
5401 CGGGACGTCG CCGAGCCGCA GCGTTCCTC CGCCGCACCA ACAGCGCCAT CCGCCGCTG  
5461 CAGGCCGCCG AAACCTTGA AAGCGCTGC GCCGCCGCGG CGCAAGAGGT GCGGAAGATT  
5521 ACCGGCTTCG ATCGGGTGAT GATCTATCGC TTCGCCTCCG ACTTCAGCGG CGAAGTGATC  
5581 GCAGAGGATC GGTGCGCCGA GGTCGAGTCA AAAGTAGGCC TGCATATCC TGCCTCAACC  
5641 GTGCCGGCGC AGGCCCGTCG GCTCTATACC ATCAACCCGG TACGGATCAT TCCCGATATC  
5701 AATTATCGGC CGGTGCCGGT CACCCAGAC CTCAATCCGG TCACCGGGCG GCCGATTGAT  
5761 CTTAGCTTCG CCATCCTGCG CAGCGTCTCG CCCGTCCATC TGAATTCAT GCGCAACATA  
5821 GGCATGCACG GCACGATGTC GATCTCGATT TTGCGCGGCG AGCGACTGTG GGGATTGATC  
5881 GTTTGCCATC ACCGAACGCC GACTACGTC GATCTCGATG GCCGCCAAGC CTGCGAGCTA  
5941 GTCGCCCAGG TTCTGGCCTG GCAGATCGGC GTGATGGAAG AGGGCGCGCC TGGAGGAGGA  
6001 GGTTCTGGTG GTGGTCACAA CAACTCCACA AAAACCAAGG AAATGTTCAT CGTGCGTGCT  
6061 CTAGAAAAGA TCCTTGCCGA TAAGGACATA CGGCGCTCCC ATCACTCGCA GCTGAAGAAG  
6121 TCCTGCGATT CGGCGCTGGA GCAGATTAAG GCGGAGCTAA TCAGTGCCGG CCAGATCGCA  
6181 GAGGGCAATG AGCTGCCCTG TGCCGCACTC CCGCTGCCCA AGAATGATGC AGCGAGCATC  
6241 ATAAATGCGG AGACCTACTT TCTCCCCTTC GAGCTTGCTT GCAAGAGCCG CTCGCCCAGG  
6301 ATCGTGGTCA CCGCACTGGA CTGCTGCAG AAACCTATTG CCTATGGCCA TTTGACAGGA  
6361 TCCATTCAGG ACTCGGCCAA TCCGGGTAC CTGCTCATCG ACCGTATCGT TGTGACCATA  
6421 TATGGCTGCT TCAGTGGTCC CCAGACGGAC GAGGCCGTCC AACTGCAGAT AATAAAGGCT  
6481 CTGCTCACGG TGGTCACCTC GCAGCATGTG GAAATCCATG AATTCACACT GCTGCAAGCT  
6541 GTGCGCACCT GCTACGACAT CTATTTGTCC AGCAAGAACC TGGTCAATCA GACCACAGCA  
6601 CGCGCTACGC TCACCCAAAT GTTGAACGTG ATATTTGCCG GCATGGAGAA TCAAGTGTAC  
6661 GAGTACCAC CTCCCAATTC CAATCCCACC AACGGCAGCA TCCACTCGGA GGATTGCAAT  
6721 GGCTCGGAG AGGAGTCGCT GCGGGATTCC GACGAAGTAA TTGCCTCGGA ACTGCTGGCG  
6781 GAGATCATAT CAGCTGCCTA CAATGAGGCG ATGAAGGATC AGGAATCGGT CCGTGAGCCA  
6841 GAGCCAACAC TTAATGGAAA CGACTACTCC TCGCACTCGG ATCAGCAGAG TGTGGAGCTG  
6901 CACAGCGAAA ACGATGCGGT TGTAACGGCT AAGTTTACGC ACATCCTGCA GAAAGATGCT  
6961 TTTCTCGTGT TCCGGGCACT GTGCAAGCTA TCGATGAAGC CTTTGCCGGA TGGACATCCA  
7021 GATCCGAAAT CGCACGAGCT GCGTTCCAAG GTGCTGTCAT TGCATCTGCT GCTGCTCATC  
7081 CTCCAGAATG CCGGGCCCGT CTTCCGCTCC AACGAGATGT TCATCATGGC CATTAAAGCAG  
7141 TACCTGTGCG TGGCCTTGTC AAACAACGGA GTCAGTCTGG TGCCGGAGGT CTTGAGCTG

7201 TCGCTTTCAA TCTTCGTTGC CCTACTCTCG AACTTCAAGG TGCATCTTAA GCGGCAGATA  
7261 GAGGTGTTCT TCAAGGAAAT CTTCTAAAC ATTCTTGAGG CGAACTCAAG CAGCTTCGAG  
7321 CACAAATGGA TGGTAATCCA AGCGCTGACA CGTATTTGTG CTGACGCCCA GTCCGTGGTG  
7381 GATATCTATG TTAATTACGA TTGCGACTTT TCGGCTGCAA ACCTTTTTGA GAGACTGGTC  
7441 AACGATCTTT CGAAAATTGC CCAGGGTCGT CAGGCTCTCG AACTGGGCGC CAATCCGATG  
7501 CAAGAGAAAT CGATGCGCAT TCGCGGCCTG GAGTGTCTTG TCTCCATTCT TAAGTGCATG  
7561 GTAGAGTGGG GTAAGGACTT GTATGTTAAT CCAAACATGC CGGTTCCACC TATGCAAGTC  
7621 CAATCGCCGA CAAGCACTGA GCAGGATCAG GCGGACACAA CTATCCAAAC GATGCACAGT  
7681 GGTTCAGTC ATAGTTTGAA CTCCAATCAG GAGCAACTAC AGGATCTTCC CGAGGCATTG  
7741 GAGGAGCGCA AGATGCGCAA GGAAGTGATG GAAACAGGCA TTGAGTTATT CAATCGTAAG  
7801 CCTCAGAAAG GAGTGCAATT CCTGCAGGAG AAGCAGTTGC TGGGTGCCAC ATGCGGGGAC  
7861 ATTGCGCGCT GGCTGCACGA GGACGAACGA CTGACAAGA CAGTGATCGG AAACACTATT  
7921 GGCGAGAATG ACGACCACTC CAAGGAAGTG ATGTGCGCTT ACATCGATGC CTTTACTTTT  
7981 GCCTAAATGG AGGTGGTGGC CGCCTTGAGA TTTCTTCTCG AGGGGTTCCG CCTGCCAGGA  
8041 GAAGCACAAA AAATCGATCG GCTGATGGAG AAGTTCGCCA GTAGATACTG CGAATGCAAT  
8101 CCGAAGAACC AGCTATTCCA AAGCGCAGAC ACCGTCTACG TGCTGGCATT CAGCATCATT  
8161 cTGCTGACCA CGGATCTTCA TTCGCCGAG GTCAAGCACA AGATGACCAA GGAGCAGTAC  
8221 ATTAATAATGA ACCGCGGCAT CAGCGACAGC AAGTCCGATT TGCCCCAGGA GTACTTGTGCG  
8281 TCCATCTACG ACGAGATTTT TGAACACGAA ATTAAGATGA AGAACAACTC CGGTATGCTT  
8341 CAACAGGCGA AACCCACTGG AAAGCAGGCC TTCATAACGG AGAAACGCAG AAAGCTGTTG  
8401 TGGAACATGG AGATGGAGGT CATCTCGCTG ACGGCCACCA ATCTAATGCA GTCAGTTTCG  
8461 CACGTCAAGT CACCCTTCAC CTCAGCGAAA CACTTGGAGC ATGTCCGGCC CATGTTCAAA  
8521 ATGGCTTGGG CACCATTCTT GGCCGCTTTC TCTGTGGGTC TCCAGGACTG CGACGATCCT  
8581 GAGATTGCTA CACTCTGCTT GGATGGTATA CGTTGTGCTA TTCGAATCGC ATGCATCTTC  
8641 CACATGTCCC TGGAGCGAGA TGCCTATGTA CAAGCCCTGG CCAGGTTTAC TCTCCTGAAT  
8701 GCTAACTCGC CCATCAACGA AATGAAGGCC AAGAATATCG ATACCATCAA GACGCTTATA  
8761 ATGGTAGCCC ACACGGATGG CAATTATCTG GGCAGCAGCT GGCTGGATAT AGTGAAGTGC  
8821 ATTAGCCAGT TGGAGCTGGC CCAACTGATC GGCAGTGGG TGCGGCCCCA GTTTCTTTCT  
8881 GGAGCGCAGA CAACGCTCAA GGAICTGCTT AATCCCAGCG TGAAAGAACA CATCGGCGAG  
8941 ACGAGCAGCC AGAGCGTGGT GGTCGAGTC GATCGTATTT TCACCGGCTC AATGCGACTG  
9001 GATGGCGATG CTATCGTGGG CTTCTGTAAG GCCCTGTGCC AGGTGTCTGT GGATGAGCTT  
9061 CAGCAGCAGC AACCGAGGAT GTTCTCCTTG CAAAAGATAG TGGAATTAG TTAACAAC  
9121 ATGGAGCGTA TTCGTCTGCA GTGGTCACGC ATTTGGCAAG TTTTGGGTGA GCACTTTAAC  
9181 GCGGTCGGAT GCAATAGCAA CGAGGAGATC TCATTTTTCG CCCTGGACTC ACTGCGTCAG  
9241 TTGTCGATGA AGTTCATGGA GAAGGGCGAG TTCAGTAATT TCCGCTTCCA GAAGGATTC  
9301 CTGCGTCCCT TTGAGCATAT CATGAAGAAA AACGCATCGC CGGCAATACG AGATATGGTG

9361	GTGCGCTGCA	TTGCCAGAT	GGTAAACTCA	CAGGCGCATA	ACATCCGTTC	CGGCTGGAAG
9421	AATATCTTTA	GCATTTTCCA	CCTGGCAGCG	GGAGACAACG	AAGAGCCAAT	TGTGGAGCTG
9481	GCCTTCCAAA	CCACGGGCAA	AATCATCGGT	GATCTGTACA	AGCGTCAGTT	CGCCATTATG
9541	GTGGACTCGT	TCCAGGATGC	GGTCAAGTGC	CTGTCAGAGT	TCGCCACCGC	CAGATTCCCC
9601	GATACCAGCA	TGGAAGCCAT	ACGTCTGGTC	CGTACCTGCG	CGCAGTGCCT	CCACGAGGCA
9661	CCACAACGTG	TTGCGGAGCA	TGCCGGCATG	GAGAACGACG	CCTCGGTGGC	CGAGGAGGAT
9721	CGAGTCTGGG	TGCGCGGCTG	GTTTCCGATG	CTATTCTCGC	TTTCTGCGT	GGTCAATCGC
9781	TGCAAATTGG	ATGTGCGTAC	TCGCGCCTTA	ACCGTGCTTT	TTGAGATTGT	GAAGACGTAT
9841	GGTGAGAGCT	TCAAGCCCCA	TTGGTGAAG	GATCTCTTCA	ATGTGATCTT	CCGTATCTTC
9901	GACAACATGA	AATTGCCGGA	GCACGTCACC	GAGAAGTCCG	AATGGATGAC	GACCACATGC
9961	AACCACGCCT	TGTACGCTAT	TATTGATGTC	TTCACGCAGT	ATTTGATGT	TCTTGGTCAT
10021	CTGCTGCTGG	AGGAGCTCTT	CGCCCAGCTG	CATTGGTGTG	TTCAGCAGAG	TAACGAGCAG
10081	TTGGCGCGAT	CTGGCACCAA	TTGCCTGGAG	AACCTCGTCA	TTTCGAATGG	ATTCAAGTTC
10141	AACGAGTCCA	CCTGGGACAA	GACGTGCCAG	TGCATCCTGG	ACATCTTCAA	CGCCACTTTG
10201	CCGCAGGATC	TCCTCAGTTG	GCGGCCGAAA	GCACATTCCA	GTAACAATAT	ACCCCAGGAG
10261	CACAACCACT	TTGAGGCGCT	GCATATCCGC	TGCGTAGTCC	AGCTGGAAct	GATACAGACC
10321	ATGGATAACA	TTGTCTTTTT	CCCGGCCACG	TCGCGCAAGG	AGGATGCCGA	AACGCTGGCC
10381	CAGGCGGCGG	CAGACTTAAC	AGGCGGCAGG	AGCGGTTCGC	AGTCGCAGCT	GCTGGAGTGC
10441	CAGCGGGAGG	AGCAGGGAAT	GTACGGCTAT	CTGAGAACCC	GCCAGCTGCT	CACCCTGGCC
10501	GACTGTCTGA	TGCAGTCGCA	CCGTTTTGCC	AAGCGCTTCA	ACGCCGATCA	CGACCAACGC
10561	AGCCTGCTTT	GGCGGGCGGG	ATTCAAGGGA	TCTGTAAAC	CGAATCTGCT	GAAGCAGGAG
10621	ACCTCGTCGC	TGGCCTGCGT	CCTGCGCATT	TTCTTCAAGA	TGTACGGCGA	CGAGAATAGA
10681	CGCAGCGATT	GGCCCAGCAT	CGAGCAGGAA	CTGGTGCAGG	TCTGCAAGGA	GGCACTGGGC
10741	TACTATTTGA	GTTTGAGAG	CGAGGCACAC	CGAGATGCGT	GGACATGCT	GCTGCTGCTC
10801	ATCCTGACGC	GCCTGCTCAA	GATGTCCGAT	GCCAGGTTCC	CCACCCACGT	TTCCAActAC
10861	TACAGCCTGC	TGTGCGAGAT	GATGTGCTTC	GACCTCAAGC	CCGAActGAG	AAGTGTCTTT
10921	AGGCGTGTGT	TCATGCGCAT	CGGTCCAGTA	TTCAATATAA	TGAGCGTTAA	ATAAttctag
10981	tcgaccatga	agatcaagat	cattgccccg	ccagagcgca	agtactctgt	ctgggccctt
11041	cgaaggtaag	cctatcccta	accctctcct	cggctctgat	tctacgcgta	ccggctatca
11101	tcaccatcac	cattgagttt	aaaccgctg	atcagcctcg	actgtgcctt	ctaagatcca
11161	gacatgataa	gatacattga	tgagtttggg	caaaccacaa	ctagaatgca	gtgaaaaaaa
11221	tgctttatatt	gtgaaatttg	tgatgctatt	gctttatttg	taaccatt	
//						
pMT-hyg-V5-iRFP713-Sec71 <sup>F713Y</sup>						
LOCUS	pMT_hyg_V5_iRFP713-Sec71 F713Y		11268 bp	ds-DNA	circular	
28-FEB-2020						

```
DEFINITION pMT-puro Sequencing Result
ORGANISM other sequences; artificial sequences; vectors.
COMMENT pMT-mAX2m-blast from 1 to 5500
COMMENT pMT-mAX2m-neo from 1 to 5813
COMMENT pMT-mAPEX2m from 1 to 5561
COMMENT pMT-mSOG1m from 1 to 5078
COMMENT pMT-mSOG4m from 1 to 5114
COMMENT ApEinfo:methylated:0
FEATURES             Location/Qualifiers
  misc_feature       4892..4909
                     /label=Metallothionein_primer
                     /ApEinfo_fwdcolor=#ff3600
                     /ApEinfo_revcolor=#ff3600
                     /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                     width 5 offset 0
  misc_feature       4927..4932
                     /label=KpnI
                     /ApEinfo_fwdcolor=#ffffcc
                     /ApEinfo_revcolor=#ffffcc
                     /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                     width 5 offset 0
  misc_feature       complement(225..1250)
                     /label=Hyg
                     /ApEinfo_fwdcolor=#ffffcc
                     /ApEinfo_revcolor=#00ff00
                     /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                     width 5 offset 0
  gene               complement(143..173)
                     /gene="puro (variant)"

/translation="MGSCAPFGRALRVVGRASGTGLAGHAPGARSFGHLDVGGDGAE
PLVEGEVAGRGLQEGGHPGALGRLHSGEHDGAAQTLALVVGRDADGGQEPRGLLGPV
RRQEAFHLLLRGQPFTAQLGHARADLGEHRPRFDALRRGPDRHRGAVVRDPHLADVEP
```

```
DAREEEFLQLGDPLDVAVRVDGVARGGVVGERGGEGAYGPGDVVAGGEAHRGLVLGHG
      RSSPCEGSRAWVRGWRRHRSWRPTCRHASYPARAARGICCNL*"
      /label=puro (variant)
      /ApEinfo_fwdcolor=#ffffcc
      /ApEinfo_revcolor=#ffffcc
      /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
      width 5 offset 0
misc_feature 95..115
      /label=New Feature
      /ApEinfo_fwdcolor=cyan
      /ApEinfo_revcolor=#00ff00
      /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
      width 5 offset 0
promoter 4463..4479
      /label=M13_forward20_primer
      /ApEinfo_fwdcolor=#ccffed
      /ApEinfo_revcolor=#ccffed
      /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
      width 5 offset 0
misc_feature 4448..4470
      /label=M13_pUC_fwd_primer
      /ApEinfo_fwdcolor=#ff3600
      /ApEinfo_revcolor=#ff3600
      /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
      width 5 offset 0
promoter complement(3926..3954)
      /label=AmpR_promoter
      /ApEinfo_fwdcolor=#ccffed
      /ApEinfo_revcolor=#ccffed
      /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
      width 5 offset 0
rep_origin complement(2250..2869)

/translation="MSIQHFRVALIPFFAAFLPVAHPETLVKVKDAEDQLGARVGY
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
```



YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLTTIGGPKELTAFLHNMGDHVTRL

DRWEPELNEAIPNDERDTTMPVAMATTLRKLTTGELLTLASRQQLIDWMEADKVAGPL

LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA

EIGASLIKHW\*"

/label=pBR322\_origin

/ApEinfo\_fwdcolor=pink

/ApEinfo\_revcolor=pink

/ApEinfo\_graphicformat=arrow\_data {{0 1 2 0 0 -1}} {{}} 0}

width 5 offset 0

CDS 6016..10971

/label=Sec71

/ApEinfo\_fwdcolor=#99ccff

/ApEinfo\_revcolor=#cde7f7

/ApEinfo\_graphicformat=arrow\_data {{0 1 2 0 0 -1}} {{}} 0}

width 5 offset 0

misc\_feature complement(1876..1898)

/translation="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGY

IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE

YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLTTIGGPKELTAFLHNMGDHVTRL

DRWEPELNEAIPNDERDTTMPVAMATTLRKLTTGELLTLASRQQLIDWMEADKVAGPL

LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA

EIGASLIKHW\*"

/label=M13\_pUC\_rev\_primer

/ApEinfo\_fwdcolor=#ff3600

/ApEinfo\_revcolor=#ff3600

/ApEinfo\_graphicformat=arrow\_data {{0 1 2 0 0 -1}} {{}} 0}

width 5 offset 0

misc\_feature complement(1350..1708)

```
    /label=Copia Promoter?  
    /ApEinfo_fwdcolor=#cde7f7  
    /ApEinfo_revcolor=#cde7f7  
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}  
width 5 offset 0  
misc_feature 10569..10569  
    /label=T>G silent  
    /ApEinfo_fwdcolor=cyan  
    /ApEinfo_revcolor=#00ff00  
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}  
width 5 offset 0  
misc_feature complement(4327..4482)  
    /label=lacZ_a  
    /ApEinfo_fwdcolor=#ff3600  
    /ApEinfo_revcolor=#ff3600  
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}  
width 5 offset 0  
misc_feature complement(4113..4135)  
    /label=pGEX_3_primer  
    /ApEinfo_fwdcolor=#ff3600  
    /ApEinfo_revcolor=#ff3600  
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}  
width 5 offset 0  
primer_bind 8772..8791  
    /label=sec71-GF6  
    /ApEinfo_fwdcolor=#ff00bd  
    /ApEinfo_revcolor=#ff0003  
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}  
width 5 offset 0  
gene complement(3024..3884)  
    /gene="Ampicillin"  
    /label=Ampicillin  
    /ApEinfo_fwdcolor=#ffffcc  
    /ApEinfo_revcolor=#ffffcc  
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}  
width 5 offset 0
```

```
promoter      complement(1912..1941)

/translation="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGY
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHVTRL
DRWEPELNEAIPNDERDTMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPL
LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
      EIGASLIKHW*"
      /label=lac_promoter
      /ApEinfo_fwdcolor=#ccffed
      /ApEinfo_revcolor=#ccffed
      /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
      width 5 offset 0
misc_feature  8631..8631
      /label=A>G in 40A
      /ApEinfo_fwdcolor=cyan
      /ApEinfo_revcolor=#00ff00
      /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
      width 5 offset 0
promoter      complement(1859..1877)

/translation="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGY
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHVTRL
DRWEPELNEAIPNDERDTMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPL
LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
      EIGASLIKHW*"
      /label=M13_reverse_primer
```

```

    /ApEinfo_fwdcolor=#ccffed
    /ApEinfo_revcolor=#ccffed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    4489..4856
    /label=MT-promoter
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
protein_bind    7735..8307
    /label=Sec7 domain
    /ApEinfo_fwdcolor=#0080ff
    /ApEinfo_revcolor=#0080ff
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
CDS            complement(4323..4391)
    /label=LacZ alpha
    /ApEinfo_fwdcolor=#6495ed
    /ApEinfo_revcolor=#6495ed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    3998..4015
    /label=pQE60-F
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    10554..10554
    /label=C>T silent
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    complement(1859..1879)
    /label=M13R_GW
```

```
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    complement(1859..1879)
    /label=M13-rev
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=green
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    8252..8275
    /label=Sec71-DRSC01893-F (8A10)
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    4462..4479
    /label=M13-fwd
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=green
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    4436..4459
    /label=M13F
    /ApEinfo_fwdcolor=#0a00ff
    /ApEinfo_revcolor=#0a00ff
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    10056..10075
    /label=sec71-GF8
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    4832..4849
    /label=SEQ-MT-F2
```

```

    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
rep_origin    complement(2247..2929)
    /label=ColE1 origin
    /ApEinfo_fwdcolor=gray50
    /ApEinfo_revcolor=gray50
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    9511..9532
    /label=sec71-GF7
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
CDS    complement(3027..3686)
    /label=AmpR
    /ApEinfo_fwdcolor=yellow
    /ApEinfo_revcolor=yellow
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_binding    complement(1885..1907)
    /label=Lac0
    /ApEinfo_fwdcolor=#6495ed
    /ApEinfo_revcolor=#6495ed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    complement(9564..9585)
    /label=Sec71-GR3
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    complement(3605..3629)
    /label=Amp-GF
```

```
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    3147..3170
    /label=Amp-GR1
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    7071..7071
    /label=C>T confirmed in 40A 2013
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    4462..4479
    /label=M13F_GW
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    1737..1759
    /label=Seq-EB-R
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    8559..8559
    /label=T>C in 40A
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    1254..1268
    /label=New Feature(1)
```

```
    /ApEinfo_label=New Feature
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature 8670..8670
    /label=A>C in 40A
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature complement(11179..11198)
    /label=EBV_rev_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature complement(11136..11153)
    /label=BGH_rev_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature 11096..11113
    /label=6xHis
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature 11045..11086
    /label=V5
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature 11013..11032
```



```

    /label=New Feature(2)
    /ApEinfo_label=New Feature
    /ApEinfo_fwdcolor=#ffffcc
    /ApEinfo_revcolor=#ffffcc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature 11033..11038
    /label=ApaI GGGCC^C
    /ApEinfo_fwdcolor=#ffffcc
    /ApEinfo_revcolor=#ffffcc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature 5995..6015
    /label=Linker GL3
    /ApEinfo_fwdcolor=#ffcc66
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature 5992..5994
    /label=Linker GL3(1)
    /ApEinfo_label=Linker GL3
    /ApEinfo_fwdcolor=#ffcc66
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature 8152..8154
    /label=S191
    /ApEinfo_fwdcolor=#66ff66
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
CDS 5035..5982
    /codon_start=1
    /product="phytochrome-based near-infrared fluorescent
protein, originally termed iRFP (Filonov et al., 2011)"
    /note="name was changed to iRFP713 (Shcherbakova and
```

```
Verkhusha, 2013)"  
  
/translation="MAEGSVARQPDLLTCDDEPIHIPGAIQPHGLLLALAADMTIVAG  
S  
DNLPELTGLAIGALIGRSAADVFDSETHNRLTIALAEPGAAVGAPITVGFTMRKDAGF  
I  
GSWHRHDQLIFLELEPPQRDVAEPQAFFRRTNSAIRRLQAAETLESACAAAAQEVRKI  
T  
GFDRVMIYRFASDFSGEVIAEDRCAEVESKLGHPASTVPAQARRLYTINPVRIIPD  
I  
NYRPVPVTPDLNPVTGRPIDLFAILRSVSPVHLEFMRNIGMGTMSISILRGERLWG  
L IVCHHRTPYYVDLDGRQACELVAQVLAWQIGVMEE"  
/label=iRFP713  
/ApEinfo_fwdcolor=#9a69fe  
/ApEinfo_revcolor=#cde7f7  
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}  
width 5 offset 0  
misc_feature 10326..10326  
/label=T>C silent  
/ApEinfo_fwdcolor=cyan  
/ApEinfo_revcolor=#00ff00  
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}  
width 5 offset 0  
misc_feature 4942..4983  
/label=V5(1)  
/ApEinfo_label=V5  
/ApEinfo_fwdcolor=#ffffcc  
/ApEinfo_revcolor=#ffffcc  
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}  
width 5 offset 0  
primer_bind complement(10226..10247)  
/label=Chang-R1
```

```

/ApEinfo_fwdcolor=#fb53d0
/ApEinfo_revcolor=#fc5a5d
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature 4984..4989
/label=SpeI
/ApEinfo_fwdcolor=#ffffcc
/ApEinfo_revcolor=#ffffcc
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind 6926..6945
/label=sec71-GF4
/ApEinfo_fwdcolor=#ff00bd
/ApEinfo_revcolor=#ff0003
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature 4990..5013
/label=Linker GL3(2)
/ApEinfo_label=Linker GL3
/ApEinfo_fwdcolor=#ffcc66
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature 8041..8043
/label=E677 (E740 in garz)
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
regulatory 5029..5038
/regulatory_class="other"
/note="vertebrate consensus sequence for strong
initiation
of translation (Kozak, 1987)"
/label=vertebrate consensus sequence for strong
initiation
```

```
of translation (Kozak, 1987)
/ApEinfo_fwdcolor=pink
/ApEinfo_revcolor=pink
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 7602..7602
/label=G>A silent
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind 8199..8217
/label=Sec71-GF11
/ApEinfo_fwdcolor=#ff00bd
/ApEinfo_revcolor=#ff0003
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind 7799..7822
/label=Sec71-GF12
/ApEinfo_fwdcolor=#ff00bd
/ApEinfo_revcolor=#ff0003
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 8161..8163
/label=M717(M194 in sec7 domain M>L = BFA-resistant)
/label=M717L atg>Ctg(BFA-resistant)
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind 7905..7927
/label=Sec71-GF14
/ApEinfo_fwdcolor=#ff00bd
/ApEinfo_revcolor=#ff0003
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
```

```
misc_feature    complement(8101..8123)
                /label=Sec71-crRNA7(Protospacer)
                /ApEinfo_fwdcolor=#ccff66
                /ApEinfo_revcolor=#ffff9f
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
misc_feature    8190..8209
                /label=Sec71-crRNA8 Protospacer
                /ApEinfo_fwdcolor=#ccff66
                /ApEinfo_revcolor=#ffffcc
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
misc_feature    8830..8830
                /label=T>C Val>Ala in FRT40A
                /ApEinfo_fwdcolor=#ccff66
                /ApEinfo_revcolor=#00ff00
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
CDS             8892..8892
                /label=Sec71-PA
                /ApEinfo_fwdcolor=#99ccff
                /ApEinfo_revcolor=#cde7f7
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
CDS             6979..6981
                /label=Sec71-PA(1)
                /ApEinfo_label=Sec71-PA
                /ApEinfo_fwdcolor=#99ccff
                /ApEinfo_revcolor=#cde7f7
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
misc_feature    10365..10365
                /label=T>C silent(1)
                /ApEinfo_label=T>C silent
                /ApEinfo_fwdcolor=cyan
                /ApEinfo_revcolor=#00ff00
```

```

    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 9511..9532
    /label=Chang-F1
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind 6570..6589
    /label=sec71-GF3
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 8892..8892
    /label=A>C silent
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
CDS 7071..7071
    /label=Sec71-PA(2)
    /ApEinfo_label=Sec71-PA
    /ApEinfo_fwdcolor=#99ccff
    /ApEinfo_revcolor=#cde7f7
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
CDS 7602..7602
    /label=Sec71-PA(3)
    /ApEinfo_label=Sec71-PA
    /ApEinfo_fwdcolor=#99ccff
    /ApEinfo_revcolor=#cde7f7
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 10449..10449
    /label=G>A silent(1)
```

```

/ApEinfo_label=G>A silent
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature 10753..10753
/label=T>C silent(2)
/ApEinfo_label=T>C silent
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind 7552..7571
/label=Sec71-GF5
/ApEinfo_fwdcolor=#ff00bd
/ApEinfo_revcolor=#ff0003
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind complement(8728..8763)
/label=Sec71-DRSC01893-R
/ApEinfo_fwdcolor=#ff00bd
/ApEinfo_revcolor=#ff0003
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature 8149..8151
/label=F190 F190Y=BFA-hypersensitive
/label=F713Y(BFA-sensitive)
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature 8721..8721
/label=A>G 40A
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
```

```
width 5 offset 0
misc_feature 10338..10338
  /label=T>C in 40A(1)
  /ApEinfo_label=T>C in 40A
  /ApEinfo_fwdcolor=cyan
  /ApEinfo_revcolor=#00ff00
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind 6042..6059
  /label=GF9
  /ApEinfo_fwdcolor=#ff00bd
  /ApEinfo_revcolor=#ff0003
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind complement(8377..8400)
  /label=Sec71-GR4
  /ApEinfo_fwdcolor=#ff00bd
  /ApEinfo_revcolor=#ff0003
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind 9139..9157
  /label=Sec71-GF13
  /ApEinfo_fwdcolor=#ff00bd
  /ApEinfo_revcolor=#ff0003
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature 10972..10974
  /label=STOP
  /ApEinfo_fwdcolor=#66ccff
  /ApEinfo_revcolor=#00ff00
  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind 8075..8096
  /label=Sec71-GF15
  /ApEinfo_fwdcolor=#fb53d0
  /ApEinfo_revcolor=#fc5a5d
```



```
      /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
      width 5 offset 0
misc_feature 8101..8103
      /label=PAM
      /ApEinfo_fwdcolor=#fc81f0
      /ApEinfo_revcolor=#fc81f0
      /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
      width 5 offset 0
misc_feature 8210..8212
      /label=PAM(1)
      /ApEinfo_label=PAM
      /ApEinfo_fwdcolor=#fc81f0
      /ApEinfo_revcolor=#fc81f0
      /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
      width 5 offset 0
primer_bind 10664..10681
      /label=Sec71-GF10
      /ApEinfo_fwdcolor=#ff00bd
      /ApEinfo_revcolor=#ff0003
      /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
      width 5 offset 0
```

ORIGIN

```
1 ataagctgca ataaacaagt tctagagtcg gtgggcctcg ggggcgggtg cggggtcggc
61 ggggccgcc cgggtggctt cggtcggagc catggggtcg tgcgctcctt tcggtcgggc
121 gctgcgggtc gtggggcggg cgtcaggcac cgggcttgcg ggtcatgcac cagttgctgg
181 ctgaggcgtt ctcgaaatca gctcttgttc ggtcggcatc tactctattc ctttgcctc
241 ggacgagtg cggggcgtcg gtttccacta tcggcgagta cttctacaca gccatcggtc
301 cagacggccg cgcttctgcg ggcgatttgt gtacgcccg cagtcccggc tccggatcgg
361 acgattgcgt cgcacgacc ctgcgccaa gctgcatcat cgaaattgcc gtcaaccaag
421 ctctgataga gttggtcaag accaatgcgg agcatatacg cccggagccg cggcgatcct
481 gcaagctccg gatgcctccg ctcgaagtag cgcgtctgct gctccataca agccaaccac
541 ggcctccaga agaagatggt ggcgacctcg tattgggaat ccccgaacat cgcctcgctc
601 cagtcaatga ccgctgttat gcggccattg tccgtcagga cattgttggg gccgaaatcc
661 gcgtgcacga ggtgccggac ttcggggcag tcctcggccc aaagcatcag ctcatcgaga
721 gcctgcgcga cggacgcact gacggtgtcg tccatcacag tttgccagtg atacacatgg
781 ggatcagcaa tcgcgcatat gaaatcacgc catgtagtgt attgaccgat tccttgcggt
```

841 ccgaatgggc cgaacccgct cgtctggcta agatcggccg cagcgatcgc atccatggcc  
901 tccgcgaccg gctgcagaac agcgggcagt tcggtttcag gcaggtcttg caacgtgaca  
961 ccctgtgcac ggcgggagat gcaataggtc aggctctcgc tgaattcccc aatgtcaagc  
1021 acttccggaa tcgggagcgc ggccgatgca aagtgccgat aaacataacg atctttgtag  
1081 aaaccatcgg cgcagctatt taccgcagc acatatccac gccctcctac atcgaagctg  
1141 aaagcacgag attcttcgcc ctccgagagc tgcacaggt cggagacgct gtcgaacttt  
1201 tcgatcagaa acttctcgac agacgtcgcg gtgagttcag gctttttcat ggtggtcgtc  
1261 tccttgtag gggtcagggg cgtgggtcag gggatggtgg cggcaccggt cgtggcggcc  
1321 gacctgcagg catgcaagct atcgaattcc tgcagcccgg gggatctggt gtaatttata  
1381 atttatattt cttttcttaa taaataaata aatagtcaag tttatgttg agttttatga  
1441 tttatatttt aagttatttc aactgcaaca ccagcaccac gacctacta cagcaaaaaa  
1501 cgtacaagaa ggaaagaagg aataaaaaga gtggtattct cttacaatat gttttatggc  
1561 ataaaaggtg tggccattca tatcaaatat aaagtagtgt tgtttaacgt tacttttcta  
1621 ggttgaatag tatattccaa cagatgatga ggggttccca atcctaaacc catttgccgt  
1681 tcccagaagc atgaaaccac cagcaccgg atcctctaga acaacaaca ttgcattcat  
1741 tttatgtttt aggttcaggg ggaggtgtgg gaggtttttt aaagcaagta aaacctctac  
1801 aatgttggtg tggctgatta tgatcagtcg acctgcaggc atgcaagctt ggcgtaatca  
1861 tggcatagc tgtttcctgt gtgaaattgt tatccgctca caattccaca caacatacga  
1921 gccggaagca taaagtgtaa agcctggggg gcctaagtag tgagctaact cacattaatt  
1981 gcgttgcgct cactgcccgc tttccagtcg ggaaacctgt cgtgccagct gcattaatga  
2041 atcggccaac gcgcggggag aggcggtttg cgtattgggc gctcttcgc ttctcgctc  
2101 actgactcgc tgcgctcggc cgttcggctg cggcgagcgg tatcagctca ctcaaaggcg  
2161 gtaatacggc tatccacaga atcaggggat aacgcaggaa agaacatgtg agcaaaaggc  
2221 cagcaaaagg ccaggaaccg taaaaggcc gcgttgctgg cgtttttcca taggctccgc  
2281 cccctgacg agcatcaca aatcgacgc tcaagtcaga ggtggcgaaa cccgacagga  
2341 ctataaagat accaggcgtt tccccctgga agctccctcg tgcgctctcc tgttccgacc  
2401 ctgccgctta ccggatacct gtccgccttt ctcccttcgg gaagcgtggc gctttctcat  
2461 agctcacgct gtaggtatct cagttcgggt taggtcgttc gctccaagct gggctgtgtg  
2521 cacgaacccc ccgttcagcc cgaccgctgc gccttatccg gtaactatcg tcttgagtcc  
2581 aacccggtg gacacgactt atcgccactg gcagcagcca ctggtaacag gattagcaga  
2641 gcgaggtatg taggcggtgc tacagagttc ttgaagtgtt ggcctaacta cggctacact  
2701 agaagaacag tatttggtat ctgcgctctg ctgaagccag ttaccttcgg aaaaagagtt  
2761 ggtagctctt gatccggcaa acaaacacc gctggtagcg gtggtttttt tgtttgcaag  
2821 cagcagatta cgcgcagaaa aaaaggatct caagaagatc ctttgatctt ttctacgggg  
2881 tctgacgctc agtgaacga aaactcacgt taagggattt tggatcatgag attatcaaaa  
2941 aggatcttca ctagatcct tttaaattaa aatgaagtt taaatcaat ctaaagtata

3001 tatgagtaaa cttggtctga cagttaccaa tgcttaatca gtgaggcacc tatctcagcg  
3061 atctgtctat ttcgttcatc catagttgcc tgactccccg tcgtgtagat aactacgata  
3121 cgggagggct taccatctgg cccagtgct gcaatgatac cgcgagacc acgctcaccg  
3181 gctccagatt tatcagcaat aaaccagcca gccggaaggg ccgagcgag aagtggctct  
3241 gcaactttat ccgcctccat ccagtctatt aattgttgcc gggagctag agtaagtagt  
3301 tcgccagtta atagtttgcg caacgttggt gccattgcta caggcatcgt ggtgtcacgc  
3361 tcgtcgtttg gtatggcttc attcagctcc ggttcccaac gatcaaggcg agttacatga  
3421 tccccatgt tgtgcaaaaa agcggtagc tccttcggtc ctccgatcgt tgtcagaagt  
3481 aagttggccg cagtgttatc actcatggtt atggcagcac tgcataattc tcttactgtc  
3541 atgccatccg taagatgctt ttctgtgact ggtgagtact caaccaagtc attctgagaa  
3601 tagtgtatgc ggcgaccgag ttgctcttgcc ccggcgtcaa tacgggataa taccgcgcca  
3661 catagcagaa ctttaaaagt gctcatcatt ggaaaacgtt cttcggggcg aaaactctca  
3721 aggatcttac cgctgttgag atccagttcg atgtaacca ctctgtcacc caactgatct  
3781 tcagcatctt ttactttcac cagcgtttct gggtgagcaa aaacaggaag gcaaaatgcc  
3841 gcaaaaaagg gaataagggc gacacggaaa tgttgaatac tcatactctt ctttttcaa  
3901 tattattgaa gcatttatca gggttattgt ctcatgagcg gatacatatt tgaatgtatt  
3961 tagaaaaata aacaaatagg ggttccgcgc acatttccc gaaaagtgcc acctgacgtc  
4021 taagaaacca ttattatcat gacattaacc tataaaaaata ggcgatcac gaggcccttt  
4081 cgtctcgcgc gtttcggtga tgacggtgaa aacctctgac acatgcagct cccggagacg  
4141 gtcacagctt gtctgtaagc ggatgccggg agcagacaag cccgtcaggg cgcgtcagcg  
4201 ggtgttggcg ggtgtcgggg ctggcttaac tatgcggcat cagagcagat tgtactgaga  
4261 gtgcaccata tgcggtgta aataccgcac agatgcgtaa ggagaaaaata ccgcatcagg  
4321 cgccattcgc cattcaggct gcgcaactgt tgggaagggc gatcgggtcg ggcctcttcg  
4381 ctattacgcc agctggcgaa agggggatgt gctgcaaggc gattaagtg ggtaacgcca  
4441 gggttttccc agtcacgacg ttgtaaaacg acggccagtg aattaattcg ttgcaggaca  
4501 ggatgtgggtg cccgatgtga ctagctcttt gctgcaggcc gtcctatcct ctggttccga  
4561 taagagacc agaactccgg cccccaccg cccaccgcca ccccatata tatgtggtac  
4621 gcaagtaaga gtgcctgcgc atgccccatg tgccccacca agagctttgc atcccatata  
4681 agtccccaaa gtggagaacc gaaccaattc ttcgcgggca gaacaaaagc ttctgcacac  
4741 gtctccactc gaatttggag ccggccggcg tgtgcaaaag aggtgaatcg aacgaaagac  
4801 ccgtgtgtaa agccgcgttt ccaaaatgta taaaaccgag agcatctggc caatgtgcat  
4861 cagttgtggt cagcagcaaa atcaagtga tcatctcagt gcaactaaag ggggatcta  
4921 gatcggggta ccgccaccAT GGGTAAGCCT ATCCCTAACC CTCTCCTCGG TCTCGATTCT  
4981 ACGACTAGTG GAGGAGGAGG TTCTGGTGGT GGTGCGGCCG CATCTGCCGC CACCATGGCT  
5041 GAAGGATCCG TCGCCAGGCA GCCTGACCTC TTGACCTGCG ACGATGAGCC GATCCATATC  
5101 CCCGGTGCCA TCCAACCGCA TGGACTGCTG CTCGCCCTCG CCGCCGACAT GACGATCGTT

5161 GCCGGCAGCG ACAACCTTCC CGAACTCACC GGACTGGCGA TCGGCGCCCT GATCGGCCGC  
5221 TCTGCGGCCG ATGTCTTCGA CTCGGAGACG CACAACCGTC TGACGATCGC CTTGGCCGAG  
5281 CCCGGGGCGG CCGTCGGAGC ACCGATCACT GTCGGCTTCA CGATGCGAAA GGACGCAGGC  
5341 TTCATCGGCT CCTGGCATCG CCATGATCAG CTCATCTTCC TCGAGCTCGA GCCTCCCCAG  
5401 CGGGACGTCG CCGAGCCGCA GGC GTTCTTC CGCCGCACCA ACAGCGCCAT CCGCCGCCTG  
5461 CAGGCCGCCG AAACCTTGA AAGCGCCTGC GCCGCCGCGG CGCAAGAGGT GCGGAAGATT  
5521 ACCGGCTTCG ATCGGGTGAT GATCTATCGC TTCGCCTCCG ACTTCAGCGG CGAAGTGATC  
5581 GCAGAGGATC GGTGCGCCGA GGTCGAGTCA AAAGTAGGCC TGCACTATCC TGCCTCAACC  
5641 GTGCCGGCGC AGGCCCGTCG GCTCTATACC ATCAACCCGG TACGGATCAT TCCCGATATC  
5701 AATTATCGGC CGGTGCCGGT CACCCAGAC CTCAATCCGG TCACCGGGCG GCGGATTGAT  
5761 CTTAGCTTCG CCATCCTGCG CAGCGTCTCG CCCGTCCATC TGGAATTCAT GCGCAACATA  
5821 GGCATGCACG GCACGATGTC GATCTCGATT TTGCGCGGCG AGCGACTGTG GGGATTGATC  
5881 GTTTGCCATC ACCGAACGCC GACTACGTC GATCTCGATG GCCGCCAAGC CTGCGAGCTA  
5941 GTCGCCCAGG TTCTGGCCTG GCAGATCGGC GTGATGGAAG AGGGCGCGCC TGGAGGAGGA  
6001 GGTTCGGTG GTGGTCACAA CAACTCCACA AAAACCAAGG AAATGTTCAT CGTGCGTGCT  
6061 CTAGAAAAGA TCCTTGCCGA TAAGGACATA CGGCGCTCCC ATCACTCGCA GCTGAAGAAG  
6121 TCCTGCGATT CGGCGCTGGA GCAGATTAAG GCGGAGCTAA TCAGTGCCGG CCAGATCGCA  
6181 GAGGGCAATG AGCTGCCCTG TGCCGCACTC CCGCTGCCCA AGAATGATGC AGCGAGCATC  
6241 ATAAATGCGG AGACCTACTT TCTCCCCTC GAGCTTGCC TCAAGAGCCG CTCGCCCAGG  
6301 ATCGTGGTCA CCGCACTGGA CTGCTGCAG AAAGTATTG CCTATGGCCA TTTGACAGGA  
6361 TCCATTCAGG ACTCGGCCAA TCCGGGTAC CTGCTCATCG ACCGTATCGT TGTGACCATA  
6421 TATGGCTGCT TCAGTGGTCC CCAGACGGAC GAGGCCGTCC AACTGCAGAT AATAAAGGCT  
6481 CTGCTCACGG TGGTCACCTC GCAGCATGTG GAAATCCATG AATTCACACT GCTGCAAGCT  
6541 GTGCGCACCT GCTACGACAT CTATTTGTCC AGCAAGAACC TGGTCAATCA GACCACAGCA  
6601 CGCGCTACGC TCACCCAAAT GTTGAACGTG ATATTTGCC GCATGGAGAA TCAAGTGTAC  
6661 GAGTACCAC CTCCCAATC CAATCCCACC AACGGCAGCA TCCACTCGGA GGATTGCAAT  
6721 GGCTCGGGAG AGGAGTCGCT GCGGGATTCC GACGAAGTAA TTGCCTCGGA ACTGCTGGCG  
6781 GAGATCATAT CAGCTGCCTA CAATGAGGCG ATGAAGGATC AGGAATCGGT CCGTGAGCCA  
6841 GAGCCAACAC TTAATGAAA CGACTACTCC TCGCACTCGG ATCACGACAG TGTGGAGCTG  
6901 CACAGCGAAA ACGATGCGGT TGTAACGGCT AAGTTTACGC ACATCCTGCA GAAAGATGCT  
6961 TTTCTCGTGT TCCGGGCACT GTGCAAGCTA TCGATGAAGC CTTTGCCGGA TGGACATCCA  
7021 GATCCGAAAT CGCACGAGCT GCGTTCCAAG GTGCTGTCAT TGCATCTGCT GCTGCTCATC  
7081 CTCCAGAATG CCGGGCCCGT CTTCCGCTCC AACGAGATGT TCATCATGGC CATTAAAGCAG  
7141 TACCTGTGCG TGGCCTTGTC AAACAACGGA GTCAGTCTGG TGCCGGAGGT CTTGAGCTG  
7201 TCGCTTTCAA TCTTCGTTGC CCTACTCTCG AACTTCAAGG TGCATCTTAA GCGGCAGATA  
7261 GAGGTGTTCT TCAAGGAAAT CTTCTAAAC ATTCTTGAGG CGAACTCAAG CAGCTTCGAG

7321 CACAAATGGA TGGTAATCCA AGCGCTGACA CGTATTTGTG CTGACGCCCA GTCCGTGGTG  
7381 GATATCTATG TTAATTACGA TTGCGACTTT TCGGCTGCAA ACCTTTTTGA GAGACTGGTC  
7441 AACGATCTTT CGAAAATTGC CCAGGGTCGT CAGGCTCTCG AACTGGGCGC CAATCCGATG  
7501 CAAGAGAAAT CGATGCGCAT TCGCGGCCTG GAGTGTCTTG TCTCCATTCT TAAGTGCATG  
7561 GTAGAGTGGG GTAAGGACTT GTATGTTAAT CCAAACATGC CGGTTCCACC TATGCAAGTC  
7621 CAATCGCCGA CAAGCACTGA GCAGGATCAG GCGGACACAA CTATCCAAAC GATGCACAGT  
7681 GGTTCAGTC ATAGTTTGAA CTCCAATCAG GAGCAACTAC AGGATCTTCC CGAGGCATTG  
7741 GAGGAGCGCA AGATGCGCAA GGAAGTGATG GAAACAGGCA TTGAGTTATT CAATCGTAAG  
7801 CCTCAGAAAG GAGTGCAATT CCTGCAGGAG AAGCAGTTGC TGGGTGCCAC ATGCGGGGAC  
7861 ATTGCGCGCT GGCTGCACGA GGACGAACGA CTGGACAAGA CAGTGATCGG AAACACTATT  
7921 GGCGAGAATG ACGACCACTC CAAGGAAGTG ATGTGCGCTT ACATCGATGC CTTTGACTTT  
7981 CGCCAAATGG AGGTGGTGGC CGCCTTGAGA TTTCTTCTCG AGGGGTTCCG CCTGCCAGGA  
8041 GAAGCACAAA AAATCGATCG GCTGATGGAG AAGTTCGCCA GTAGATACTG CGAATGCAAT  
8101 CCGAAGAACC AGCTATTCCA AAGCGCAGAC ACCGTCTACG TGCTGGCAt<sub>α</sub> CAGCATCATT  
8161 ATGCTGACCA CGGATCTTCA TTCGCCGAG GTCAAGCACA AGATGACCAA GGAGCAGTAC  
8221 ATTA<sup>4</sup>AAATGA ACCGCGGCAT CAGCGACAGC AAGTCCGATT TGCCCCAGGA GTACTTGTCTG  
8281 TCCATCTACG ACGAGATTTT TGAACACGAA ATTAAGATGA AGAACA<sup>2</sup>ACTC CGGTATGCTT  
8341 CAACAGGCGA AACCCACTGG AAAGCAGGCC TTCATAACGG AGAAACGCAG AAAGCTGTTG  
8401 TGGAACATGG AGATGGAGGT CATCTCGCTG ACGGCCACCA ATCTAATGCA GTCAGTTTCG  
8461 CACGTCAAGT CACCCTT<sup>2</sup>CAC CTCAGCGAAA CACTTGGAGC ATGTCCGGCC CATGTTCA<sup>3</sup>AA  
8521 ATGGCTT<sup>2</sup>GGA CACCATT<sup>2</sup>TCT GGCCGCTTTC TCTGTGGGTC TCCAGGACTG CGACGATCCT  
8581 GAGATTGCTA CACTCTGCTT GGATGGTATA CGTTGTGCTA TTCGAATCGC ATGCATCTTC  
8641 CACATGTCCC TGGAGCGAGA TGCCTATGTA CAAGCCCTGG CCAGGTTTAC TCTCCTGAAT  
8701 GCTAACTCGC CCATCAACGA AATGAAGGCC AAGAATATCG ATACCATCAA GACGCTTATA  
8761 ATGGTAGCCC ACACGGATGG CAATTATCTG GGCAGCAGCT GGCTGGATAT AGTGAAGTGC  
8821 ATTAGCCAGT TGGAGCTGGC CCAACTGATC GGC<sup>2</sup>ACTGGGG TGCGGCCCCA GTTCTTTTCT  
8881 GGAGCGCAGA CAACGCTCAA GGA<sup>2</sup>CTCGCTT AATCCAGCG TGAAAGAACA CATCGGCGAG  
8941 ACGAGCAGCC AGAGCGTGGT GGTCGAGTC GATCGTATTT TCACCGGCTC AATGCGACTG  
9001 GATGGCGATG CTATCGTGGG CTTCTGTAAG GCCCTGTGCC AGGTGTCTGT GGATGAGCTT  
9061 CAGCAGCAGC AACCGAGGAT GTTCTCCTTG CAAAAGATAG TGGAAATTAG TTA<sup>2</sup>CTACAAC  
9121 ATGGAGCGTA TTCGTCTGCA GTGGTCACGC ATTTGGCAAG TTTTGGGTGA GCACTTTAAC  
9181 GCGGTCGGAT GCAATAGCAA CGAGGAGATC TCATTTTTCG CCCTGGACTC ACTGCGTCAG  
9241 TTGTGATGTA AGTTCATGGA GAAGGGCGAG TTCAGTAATT TCCGCTTCCA GAAGGATTTCT  
9301 CTGCGTCCCT TTGAGCATAT CATGAAGAAA AACGCATCGC CGGCAATACG AGATATGGTG  
9361 GTGCGCTGCA TTGCCAGAT GGTA<sup>2</sup>AACTCA CAGGCGCATA ACATCCGTTCT CGGCTGGAAG  
9421 AATATCTTTA GCATTTTCCA CCTGGCAGCG GGAGACAACG AAGAGCCAAT TGTGGAGCTG

<p>9481 GCCTTCCAAA CCACGGGCAA AATCATCGGT GATCTGTACA AGCGTCAGTT CGCCATTATG            9541 GTGGACTCGT TCCAGGATGC GGTCAAGTGC CTGTCAGAGT TCGCCACCGC CAGATTCCCC            9601 GATACCAGCA TGGAAGCCAT ACGTCTGGTC CGTACCTGCG CGCAGTGCCT CCACGAGGCA            9661 CCACAACGTG TTGCGGAGCA TGCCGGCATG GAGAACGACG CCTCGGTGGC CGAGGAGGAT            9721 CGAGTCTGGG TGC GCGGCTG GTTTCCGATG CTATTCTCGC TTTCTGCGT GGTCAATCGC            9781 TGCAAATTGG ATGTGCGTAC TCGCGCCTTA ACCGTGCTTT TTGAGATTGT GAAGACGTAT            9841 GGTGAGAGCT TCAAGCCCCA TTGGTGAAG GATCTCTTCA ATGTGATCTT CCGTATCTTC            9901 GACAACATGA AATTGCCGGA GCACGTCACC GAGAAGTCCG AATGGATGAC GACCACATGC            9961 AACACGCCT TGTACGCTAT TATTGATGTC TTCACGCAGT ATTTGATGT TCTTGGTCAT            10021 CTGCTGCTGG AGGAGCTCTT CGCCCAGCTG CATTGGTGTG TTCAGCAGAG TAACGAGCAG            10081 TTGGCGCGAT CTGGCACCAA TTGCTGGAG AACCTCGTCA TTTCGAATGG ATTCAAGTTC            10141 AACGAGTCCA CCTGGGACAA GACGTGCCAG TGCATCCTGG ACATCTTCAA CGCCACTTTG            10201 CCGCAGGATC TCCTCAGTTG GCGGCCGAAA GCACATTCCA GTAACAATAT ACCCCAGGAG            10261 CACAACCACT TTGAGGCGCT GCATATCCGC TGCGTAGTCC AGCTGGAAC TATACAGACC            10321 ATGGATAACA TTGTCTTTTT CCCGGCCACG TCGCGCAAGG AGGATGCCGA AACGCTGGCC            10381 CAGCGGCGG CAGACTTAAC AGGCGGCAGG AGCGGTTGCG AGTCGCAGCT GCTGGAGTGC            10441 CAGCGGGAGG AGCAGGGAAT GTACGGCTAT CTGAGAACCC GCCAGCTGCT CACCCTGGCC            10501 GACTGTCTGA TGCAGTCGCA CCGTTTTGCC AAGCGCTTCA ACGCCGATCA CGACCAACGC            10561 AGCCTGCTTT GCGGGCGGG ATTCAAGGGA TCTGTAAAC CGAATCTGCT GAAGCAGGAG            10621 ACCTCGTCGC TGGCCTGCGT CCTGCGCATT TTCTTCAAGA TGTACGGCGA CGAGAATAGA            10681 CGCAGCGATT GGCCCGCAT CGAGCAGGAA CTGGTGCAGG TCTGCAAGGA GGCCTGGGC            10741 TACTATTTGA GTTTGAGAG CGAGGCACAC CGAGATGCGT GGACATCGCT GCTGCTGCTC            10801 ATCCTGACGC GCCTGCTCAA GATGTCCGAT GCCAGGTTG CCACCCACGT TTCCAAC TAC            10861 TACAGCCTGC TGTGCGAGAT GATGTGCTTC GACCTCAAGC CCGAAGT GAG AAGTGTCTT            10921 AGGCGTGTGT TCATGCGCAT CGGTCCAGTA TTCAATATAA TGAGCGTTAA ATAAttctag            10981 tcgaccatga agatcaagat cattgccccg ccagagcgca agtactctgt ctgggccctt            11041 cgaaggtaa cctatcccta accctctcct cggctctgat tctacgcgta ccggtcatca            11101 tcaccatcac cattgagttt aaaccgctg atcagcctcg actgtgcctt ctaagatcca            11161 gacatgataa gatacattga tgagtttggg caaaccacaa ctagaatgca gtgaaaaaaaa            11221 tgctttatatt gtgaaatttg tgatgctatt gctttatttg taaccatt</p>					
//					
CMV-ManII::EGFP					
LOCUS	CMV_ManII_EGFP	5048 bp	ds-DNA	circular	28-FEB-2020
DEFINITION	LAMP1-mGFP Sequencing Result				
ORGANISM	other sequences; artificial sequences; vectors.				

```
COMMENT    CMV-ST-EGFP from 1 to 4850
COMMENT    LAMP1_mGFP(34831) from 1 to 6015
COMMENT    ApEinfo:methylated:1
FEATURES   Location/Qualifiers
    promoter      17..569
                /label=CMV_imnearly_promoter
                /ApEinfo_fwdcolor=#ccffed
                /ApEinfo_revcolor=#ccffed
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
    misc_feature  526..546
                /label=CMV_fwd_primer
                /ApEinfo_fwdcolor=#ffcc66
                /ApEinfo_revcolor=#ffcc66
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
    promoter      527..596
                /label=CMV_promoter
                /ApEinfo_fwdcolor=#ccffed
                /ApEinfo_revcolor=#ccffed
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
    misc_feature  1934..1953

/translation="MAAPGSARRPLLLLLLLLLLGLMHCASAAMFMVKNNGTACIMA
NFSAAFVNYDTKSGPKNMTFDLPSDATVVLNRSSCGKENTSDPSLVIAFGRGHTLTL
NFTRNATRYSVQLMSFVYNLSDTHLFPNASSKEIKTVESITDIRADIDKKYRCVSGTQ
VHMNVTVTLHDATIQAYLSNSSFSRGETRCEQDRPSPPTAPPAPPSPSPSPVPKSPS
VDKYNVSGTNGTCLLASMGLQLNLTYERKDNTTVTRLLNINPNKTSASGSCGAHLVTL
ELHSEGTTVLLFQFGMNASSRFFLQGIQLNTILPDARDPAFKAANGSLRALQATVGN
```

```
SYKCNAEEHVRVTKAFSVNIFKVVWQAFKVEGGQFGSVEECLLDENSMLIPIAVGGAL  
  
AGLVLIVLIAYLVGRKRSHAGYQTIGSTGSTGSTGAVDGTAGPGSIATMVSKGEELFT  
  
GVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTCLKFICTTGKLPVPWPTLVTTLTYG  
  
VQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIEL  
  
KGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQ  
  
QNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMLKEFVTAAGITLGMDELYK*"
    /label=EBV_rev_primer
    /ApEinfo_fwdcolor=#ffcc66
    /ApEinfo_revcolor=#ffcc66
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
    rep_origin    complement(2103..2409)

/translation="MAAPGSARRPLLLLLLLLLGLMHCSAAMFMVKNNGTACIMA  
  
NFSAAFVNYDTKSGPKNMTFDLPSDATVVLNRSSCGKENTSDPSLVIAFGRGHTLTL  
  
NFTRNATRYSVQLMSFVYNLSDTHLFPNASSKEIKTVESITDIRADIDKKYRCVSGTQ  
  
VHMNNVTVLHDATIQAYLSNSSFSRGETRCEQDRPSPPTAPPAPPSPSPSPVPKSPS  
  
VDKYNVSGTNGTCLLASMGLQLNLTYERKDNTTVTRLLNINPNKTSASGSCGAHLVTL  
  
ELHSEGTTVLLFQFGMNASSRFFLQGIQLNTILPDARDPAFKAANGSLRALQATVGN  
  
SYKCNAEEHVRVTKAFSVNIFKVVWQAFKVEGGQFGSVEECLLDENSMLIPIAVGGAL  
  
AGLVLIVLIAYLVGRKRSHAGYQTIGSTGSTGSTGAVDGTAGPGSIATMVSKGEELFT  
  
GVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTCLKFICTTGKLPVPWPTLVTTLTYG
```



```
VQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIEL

KGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQ

QNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMLKEFVTAAGITLGMDELYK*"
    /label=f1_origin
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#cccccc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
promoter      2488..2516

/translation="MAAPGSARRPLLLLLLLLLLLGLMHCASAAMFMVKNNGTACIMA

NFSAAFVNYDTKSGPKNMTFDLPDATVVLNRSSCGKENTSDPSLVIAFGRGHTLTL

NFTRNATRYSVQLMSFVYNLSDTHLFPNASSKEIKTVESITDIRADIDKKYRCVSGTQ

VHMNNVTVLHDATIQAYLSNSSFSRGETRCEQDRPSPTTAPPAPPSPSPSPVPKSPS

VDKYNVSGTNGTCLLASMGLQLNLTYERKDNTTVTRLLNINPNKTSASGSCGAHLVTL

ELHSEGTTVLLFQFGMNASSRFFLQGIQLNTILPDARDPAFKAANGSLRALQATVGN

SYKCNAEEHVRVTKAFSVNIFKVVVQAFKVEGGQFGSVEECLLDENSMLIPIAVGGAL

AGLVLIVLIAYLVGRKRSHAGYQTIGSTGSTGSTGAVDGTAGPGSIATMVSKEELFT

GVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTCLKFICTTGKLPVPWPTLVTTLYG

VQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIEL

KGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQ

QNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMLKEFVTAAGITLGMDELYK*"
    /label=AmpR_promoter
```

```

    /ApEinfo_fwdcolor=#ccffed
    /ApEinfo_revcolor=#ccffed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    complement(2582..2602)

/translation="MAAPGSARRPLLLLLLLLLLLGLMHCASAAMFMVKNNGTACIMA
NFSAAFVNYDTKSGPKNMTFDLPSDATVVLNRSSCGKENTSDPSLVIAFGRGHTLTL
NFTRNATRYSVQLMSFVYNLSDTHLFPNASSKEIKTVESITDIRADIDKKYRCVSGTQ
VHMNNVTVLHDATIQAAYLSNSSFSRGETRCEQDRPSPTTAPPAPPSPSPSPVPKSPS
VDKYNVSGTNGTCLLASMGLQLNLTYERKDNTTVTRLLNINPNKTSASGSCGAHLVTL
ELHSEGTTVLLFQFGMNASSRFFLQGIQLNTILPDARDPAFKAANGSLRALQATVGN
SYKCNAEEHVRVTKAFSVNIFKVVWQAFKVEGGQFGSVEECLLDENSMLIPIAVGGAL
AGLVLIVLIAAYLVGRKRSHAGYQTIGSTGSTGSTGAVDGTAGPGSIATMVSKEELFT
GVVPILVELDGDVNGHKFSVSGEGDATYGKLTCLKFICTTGKLPVPWPTLVTTLYG
VQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIEL
KGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQ
QNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMLKEFVTAAGITLGMDELYK*"
    /label=pBABE_3_primer
    /ApEinfo_fwdcolor=#ffcc66
    /ApEinfo_revcolor=#ffcc66
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    complement(2588..2803)
```

```
/translation="MAAPGSARRPLLLLLLLLLGLMHCAAMFMVKNNGTACIMA  
NFSAAFVNYDTKSGPKNMTFDLPSDATVVLNRSSCGKENTSDPSLVIAFGRGHTLTL  
NFTRNATRYSVQLMSFVYNLSDTHLFPNASSKEIKTVESITDIRADIDKKYRCVSGTQ  
VHMNNVTVLHDATIQAYLSNSSFSRGETRCEQDRPSPTTAPPAPPSPSPSPVPKSPS  
VDKYNVSGTNGTCLLASMGLQLNLTYERKDNTTVTRLLNINPNKTSASGSCGAHLVTL  
ELHSEGTTVLLFQFGMNASSRFFLQGIQLNTILPDARDPAFKAANGSLRALQATVGN  
SYKCNAEEHVRVTKAFSVNIFKVVWQAFKVEGGQFGSVEECLLDENSMLIPIAVGGAL  
AGLVLIVLIAAYLVGRKRSHAGYQTIGSTGSTGSTGAVDGTAGPGSIATMVSKEELFT  
GVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTCLKFICTTGKLPVPWPTLVTTLYG  
VQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIEL  
KGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQ  
QNTPIGDGPVLLPDNYLSTQSALSKDPNEKRDHMLKEFVTAAGITLGMDELYK*"  
    /label=SV40_enhancer  
    /ApEinfo_fwdcolor=#ffcc66  
    /ApEinfo_revcolor=#ffcc66  
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}  
    width 5 offset 0  
promoter      2600..2868  
  
/translation="MAAPGSARRPLLLLLLLLLGLMHCAAMFMVKNNGTACIMA  
NFSAAFVNYDTKSGPKNMTFDLPSDATVVLNRSSCGKENTSDPSLVIAFGRGHTLTL  
NFTRNATRYSVQLMSFVYNLSDTHLFPNASSKEIKTVESITDIRADIDKKYRCVSGTQ
```

```
VHMNNVTVLHDATIQAYLSNSSFSRGETRCEQDRPSPTTAPPAPSPSPSPVPKSPS
VDKYNVSGTNGTCLLASMGLQLNLTYERKDNTTVTRLLNINPNKTSASGSCGAHLVTL
ELHSEGTTVLLFQFGMNASSRFFLQGIQLNTILPDARDPAFKAANGSLRALQATVGN
SYKCNAEEHVRVTKAFSVNIFKVVWQAFKVEGGQFGSVEECLLDENSMLIPIAVGGAL
AGLVLIVLIAYLVGRKRSHAGYQTIGSTGSTGSTGAVDGTAGPGSIATMVSKGEELFT
GVVPILVELDGDVNGHKFSVSGEGEGDATYKGLTLKFICTTGKLPVPWPTLVTTLTYG
VQCFSRYPDHMKQHDFKFSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIEL
KGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQ
QNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMLKEFVTAAGITLGMDELYK*"
    /label=SV40_promoter
    /ApEinfo_fwdcolor=#ccffed
    /ApEinfo_revcolor=#ccffed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
    rep_origin    2767..2844

/translation="MAAPGSARRPLLLLLLLLLLLGLMHCASAAMFMVKNNGTACIMA
NFSAAFVNYDTKSGPKNMTFDLPSDATVVLNRSSCGKENTSDPSLVIAFGRGHTLTL
NFTRNATRYSVQLMSFVYNLSDTHLFPNASSKEIKTVESITDIRADIDKKYRCVSGTQ
VHMNNVTVLHDATIQAYLSNSSFSRGETRCEQDRPSPTTAPPAPSPSPSPVPKSPS
VDKYNVSGTNGTCLLASMGLQLNLTYERKDNTTVTRLLNINPNKTSASGSCGAHLVTL
ELHSEGTTVLLFQFGMNASSRFFLQGIQLNTILPDARDPAFKAANGSLRALQATVGN
```

```
SYKCNAEEHVRVTKAFSVNIFKVVWQAFKVEGGQFGSVEECLLDENSMLIPIAVGGAL  
  
AGLVLIVLIAYLVGRKRSHAGYQTIGSTGSTGSTGAVDGTAGPGSIATMVSKGEELFT  
  
GVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTCLKFICTTGKLPVPWPTLVTTLTYG  
  
VQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIEL  
  
KGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQ  
  
QNTPIGDGPVLLPDNYLSTQSALSKDPNEKRDHMLKEFVTAAGITLGMDELYK*"  
    /label=SV40_origin  
    /ApEinfo_fwdcolor=cyan  
    /ApEinfo_revcolor=#00ff00  
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}  
    width 5 offset 0  
    misc_feature    2829..2848  
  
/translation="MAAPGSARRPLLLLLLLLLLLGLMHCASAAMFMVKNNGTACIMA  
  
NFSAAFSVNYDTKSGPKNMTFDLPSDATVVLNRSSCGKENTSDPSLVIAFGRGHTLTL  
  
NFTRNATRYSVQLMSFVYNLSDTHLFPNASSKEIKTVESITDIRADIDKKYRCVSGTQ  
  
VHMNNVTVLHDATIQAYLSNSSFSRGETRCEQDRPSPPTAPPAPPSPSPSPVPKSPS  
  
VDKYNVSGTNGTCLLASMGLQLNLTYERKDNTTVTRLLNINPNKTSASGSCGAHLVTL  
  
ELHSEGTTVLLFQFGMNASSRFFLQGIQLNTILPDARDPAFKAANGSLRALQATVGN  
  
SYKCNAEEHVRVTKAFSVNIFKVVWQAFKVEGGQFGSVEECLLDENSMLIPIAVGGAL  
  
AGLVLIVLIAYLVGRKRSHAGYQTIGSTGSTGSTGAVDGTAGPGSIATMVSKGEELFT  
  
GVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTCLKFICTTGKLPVPWPTLVTTLTYG
```

```
VQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIEL
KGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQ
QNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMLKEFVTAAGITLGMDELYK*"
    /label=SV40pro_F_primer
    /ApEinfo_fwdcolor=#ffcc66
    /ApEinfo_revcolor=#ffcc66
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
CDS      2951..3745
/translation="MIEQDGLHAGSPAAWVERLFGYDWAQQTIGCSDAAVFRLSAQGR
PVLVFKTDLSGALNELQDEAARLSWLATTGVPCAAVLDVVTEAGRDWLLLGEVPGQDL
LSSHLAPAEEKVSIMADAMRRLHTLDPATCPFDHQAKHRIERARTRMEAGLVDQDDLDE
EHQGLAPAEELFARLKASMPDGEDLVVTHGDACLPNIMVENGRFSGFIDCGRLGVADRY
    QDIALATRDIAEELGGEWADRFLVLYGIAAPDSQRIAFYRLLDEFF*"
    /label=ORF frame 3
    /ApEinfo_fwdcolor=#99ccff
    /ApEinfo_revcolor=#99ccff
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
gene     2954..3742
    /gene="NeoR/KanR"
/translation="MIEQDGLHAGSPAAWVERLFGYDWAQQTIGCSDAAVFRLSAQGR
PVLVFKTDLSGALNELQDEAARLSWLATTGVPCAAVLDVVTEAGRDWLLLGEVPGQDL
LSSHLAPAEEKVSIMADAMRRLHTLDPATCPFDHQAKHRIERARTRMEAGLVDQDDLDE
EHQGLAPAEELFARLKASMPDGEDLVVTHGDACLPNIMVENGRFSGFIDCGRLGVADRY
    QDIALATRDIAEELGGEWADRFLVLYGIAAPDSQRIAFYRLLDEFF*"
```

```

        /label=NeoR/KanR
        /ApEinfo_fwdcolor=#ffffcc
        /ApEinfo_revcolor=#ffffcc
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
CDS      complement(3260..3796)

/translation="MAGWASLGRSFRTPEsrSEELVKKaIEGDALRIGSGDtvKHEEA
VSPFAAKLFSNITGSQRYVLIaVRHTQPATVDESrKAAIFHHDIRQAGIAMGHDEILA
VGHARLEPGEQFGWREPLMLFVQIILIDKTGFHPSTCSLDAMFRLVVEWAGSRiKRMQ
        PPHCISHDGYFLGRSKVR*"
        /label=ORF frame 2
        /ApEinfo_fwdcolor=#99ccff
        /ApEinfo_revcolor=#99ccff
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
terminator      3920..4189

/translation="MAGWASLGRSFRTPEsrSEELVKKaIEGDALRIGSGDtvKHEEA
VSPFAAKLFSNITGSQRYVLIaVRHTQPATVDESrKAAIFHHDIRQAGIAMGHDEILA
VGHARLEPGEQFGWREPLMLFVQIILIDKTGFHPSTCSLDAMFRLVVEWAGSRiKRMQ
        PPHCISHDGYFLGRSKVR*"
        /label=TK_PA_terminator
        /ApEinfo_fwdcolor=pink
        /ApEinfo_revcolor=pink
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
rep_origin      4337..4956

/translation="MAGWASLGRSFRTPEsrSEELVKKaIEGDALRIGSGDtvKHEEA
VSPFAAKLFSNITGSQRYVLIaVRHTQPATVDESrKAAIFHHDIRQAGIAMGHDEILA
```

```
VGHARLEPGEQFGWREPLMLFVQIILIDKTGFHPSTCSLDAMFRLVVEWAGSRIKRMQ
      PPHCISHDGYFLGRSKVR*"
      /label=pBR322_origin
      /ApEinfo_fwdcolor=cyan
      /ApEinfo_revcolor=#00ff00
      /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
      width 5 offset 0
gene      1004..1717
      /gene="EGFP"

/translation="MVSKGEELFTGVVPIVELDGDVNGHKFSVSGEGEGDATYGKLT
LKFICTTGKLPVPWPTLVTTLTYGVCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFK
DDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNNSHNVYIMADKQKN

GIKVNFKIRHNIEDGSVQLADHYQNTPIGDGPVLLPDNHYLSTQSALS KDPNEKRDH
      MVLLEFVTAAGITLGMDELYKSGLRSAQASNSAVDGTAGPGSTGSR*"
      /label=EGFP
      /ApEinfo_fwdcolor=#66ff66
      /ApEinfo_revcolor=#ffffcc
      /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
      width 5 offset 0
misc_feature      complement(1046..1067)

/translation="MVSKGEELFTGVVPIVELDGDVNGHKFSVSGEGEGDATYGKLT
LKFICTTGKLPVPWPTLVTTLTYGVCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFK
DDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNNSHNVYIMADKQKN

GIKVNFKIRHNIEDGSVQLADHYQNTPIGDGPVLLPDNHYLSTQSALS KDPNEKRDH
      MVLLEFVTAAGITLGMDELYKSGLRSAQASNSAVDGTAGPGSTGSR*"
      /label=EGFP_N_primer
      /ApEinfo_fwdcolor=#ffcc66
```



```

    /ApEinfo_revcolor=#ffcc66
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1} {} 0}
    width 5 offset 0
misc_feature    1184..1213

/translation="MVSKGEELFTGVVPIVELDGDVNGHKFSVSGEGEGDATYGKLT
LKFICTTGKLPVPWPTLVTTLTYGVCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFK
DDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNNSHNVYIMADKQKN

GIKVNFKIRHNIEDGSVQLADHYQNTPIGDGPVLLPDNHYLSTQSALS KDPNEKRDH
    MVLLEFVTAAGITLGMDELYKSGLRSAQASNSAVDGTAGPGSTGSR*"
    /label=Y66 (EGFP)
    /ApEinfo_fwdcolor=#ffcc66
    /ApEinfo_revcolor=#ffcc66
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1} {} 0}
    width 5 offset 0
misc_feature    1654..1675

/translation="MVSKGEELFTGVVPIVELDGDVNGHKFSVSGEGEGDATYGKLT
LKFICTTGKLPVPWPTLVTTLTYGVCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFK
DDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNNSHNVYIMADKQKN

GIKVNFKIRHNIEDGSVQLADHYQNTPIGDGPVLLPDNHYLSTQSALS KDPNEKRDH
    MVLLEFVTAAGITLGMDELYKSGLRSAQASNSAVDGTAGPGSTGSR*"
    /label=EGFP_C_primer
    /ApEinfo_fwdcolor=#ffcc66
    /ApEinfo_revcolor=#ffcc66
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1} {} 0}
    width 5 offset 0
misc_feature    635..982
    /label=ManII mouse
    /ApEinfo_fwdcolor=cyan
```

/ApEinfo\_revcolor=#00ff00

/ApEinfo\_graphicformat=arrow\_data {{0 1 2 0 0 -1} {} 0}

width 5 offset 0

ORIGIN

1 catgcattag ttattaatag taatcaatta cggggtcatt agttcatagc ccatatatgg  
61 agttccgcgt tacataactt acggtaaatg gcccgcctgg ctgaccgcc aacgaccccc  
121 gccattgac gtcaataatg acgtatgttc ccatagtaac gccaataggg actttccatt  
181 gacgtcaatg ggtggagtat ttacggtaaa ctgcccactt ggcagtacat caagtgtatc  
241 atatgccaag tacgccccct attgacgtca atgacggtaa atggcccgcc tggcattatg  
301 cccagtacat gaccttatgg gactttccta cttggcagta catctacgta ttagtcatcg  
361 ctattacat ggtgatgcgg ttttggcagt acatcaatgg gcgtggatag cggtttgact  
421 cacggggatt tccaagtctc caccctattg acgtcaatgg gagtttgttt tggcaccaaa  
481 atcaacggga ctttcaaaa tgctgtaaca actccgccc attgacgcaa atgggcggta  
541 ggcgtgtacg gtgggaggtc tatataagca gagctggttt agtgaaccgt cagatccgct  
601 agcgtaccg gactcagatc tcgagctgc caccATGAAG TTAAGTCGCC AGTTCACCGT  
661 GTTTGGCAGC GCGATCTTCT GCGTCGTAAT CTTCTCACTC TACCTGATGC TGGACAGGGG  
721 TCACTTGAC TACCCTCGGG GCCCGGCCA GGAGGGCTCC TTTCCGCAGG GCCAGCTTTC  
781 AATATTGCAA GAAAAGATTG ACCATTTGGA GCGTTTGCTC GCTGAGAACA ACGAGATTAT  
841 CTCAAATATC AGAGACTCAG TCATCAACCT GAGCGAGTCT GTGGAGGACG GCCCGCGGGG  
901 GTCACCAGGC AACGCCAGCC AAGGCTCCAT CCACCTCCAC TCGCCACAGT TGGCCCTGCA  
961 GGCTGACCCC AGAGACTGTT TGGATCCACC GGTcgccacc ATGGTGAGCA AGGGCGAGGA  
1021 GCTGTTACCC GGGGTGGTGC CCATCCTGGT CGAGCTGGAC GGCGACGTAA ACGGCCACAA  
1081 GTTCAGCGTG TCCGGCGAGG GCGAGGGCGA TGCCACCTAC GGCAAGCTGA CCCTGAAGTT  
1141 CATCTGCACC ACCGGCAAGC TGCCCGTGCC CTGGCCCACC CTCGTGACCA CCCTGACCTA  
1201 CGGCGTGACG TGCTTCAGCC GCTACCCCGA CCACATGAAG CAGCACGACT TCTTCAAGTC  
1261 CGCCATGCCC GAAGGCTACG TCCAGGAGCG CACCATCTTC TTCAAGGACG ACGGCAACTA  
1321 CAAGACCCGC GCCGAGGTGA AGTTCGAGGG CGACACCCTG GTGAACCGCA TCGAGCTGAA  
1381 GGGCATCGAC TTCAAGGAGG ACGGCAACAT CCTGGGGCAC AAGCTGGAGT ACAACTACAA  
1441 CAGCCACAAC GTCTATATCA TGGCCGACAA GCAGAAGAAC GGCATCAAGG TGAACCTCAA  
1501 GATCCGCCAC AACATCGAGG ACGGCAGCGT GCAGCTCGCC GACCACTACC AGCAGAACAC  
1561 CCCCATCGGC GACGGCCCCG TGCTGCTGCC CGACAACCAC TACCTGAGCA CCCAGTCCGC  
1621 CCTGAGCAAA GACCCCAACG AGAAGCGCGA TCACATGGTC CTGCTGGAGT TCGTGACCGC  
1681 CGCCGGGATC ACTCTCGGCA TGGACGAGCT GTACAAGTAA agcggccgcg actctagatc  
1741 ataatcagcc ataccacatt tgtagagggt ttacttgctt taaaaaacct cccacacctc  
1801 ccctgaacc tgaaacataa aatgaatgca attgttgttg ttaacttgtt tattgcagct  
1861 tataatgggtt acaataaag caatagcatc acaatttca caaataaagc atttttttca

1921 ctgcattcta gttgtggttt gtccaaactc atcaatgtat cttaaggcgt aaattgtaag  
1981 cgtaaatatt ttgttaaaat tcgcgttaaa tttttgttaa atcagctcat tttttaacca  
2041 ataggccgaa atcggcaaaa tcccttataa atcaaaagaa tagaccgaga tagggttgag  
2101 tgttgttcca gtttggaaca agagtccact attaaagaac gtggactcca acgtcaaagg  
2161 gcgaaaaacc gtctatcagg gcgatggccc actacgtgaa ccatcacctt aatcaagttt  
2221 tttggggctc aggtgccgta aagcactaaa tcggaaccct aaagggagcc cccgatttag  
2281 agcttgacgg ggaagccgg cgaacgtggc gagaaaggaa ggaagaaag cgaaaggagc  
2341 gggcgctagg gcgctggcaa gtgtagcggc cacgctgcgc gtaaccacca caccgccgc  
2401 gcttaatgcg ccgctacagg gcgctcagg tggcactttt cggggaaatg tgcgcggaac  
2461 ccctatattg ttatatttct aaatacattc aaatatgtat ccgctcatga gacaataacc  
2521 ctgataaatg cttcaataat attgaaaaag gaagagtcct gaggcggaaa gaaccagctg  
2581 tggaaatgtg gtcagttagg gtgtggaaag tccccaggct cccagcagg cagaagtatg  
2641 caaagcatgc atctcaatta gtcagcaacc aggtgtggaa agtccccagg ctccccagca  
2701 ggcagaagta tgcaaagcat gcatctcaat tagtcagcaa ccatagtccc gccctaact  
2761 ccgcccattc cgcccctaac tccgcccagt tccgcccatt ctccgcccga tggctgacta  
2821 atttttttta tttatgcaga ggccgaggcc gcctcggcct ctgagctatt ccagaagtag  
2881 tgaggaggct tttttggagg cctaggcttt tgcaaagatc gatcaagaga caggatgagg  
2941 atcgtttcgc atgattgaac aagatggatt gcacgcaggc tctccggccg cttgggtgga  
3001 gaggctattc ggctatgact gggcacaaca gacaatcggc tgctctgatg ccgccgtgtt  
3061 ccggctgtca gcgcaggggc gcccggttct ttttgtcaag accgacctgt ccggtgcctt  
3121 gaatgaactg caagacgagg cagcgcggct atcgtggctg gccacgacgg gcgttccttg  
3181 gcgagctgtg ctcgacgttg tcaactgaag ggaagggac tggctgctat tgggcgaagt  
3241 gccggggcag gatctcctgt catctcacct tgctcctgcc gagaaagat ccatcatggc  
3301 tgatgcaatg cggcggctgc atacgcttga tccggctacc tgcccattcg accaccaagc  
3361 gaaacatcgc atcagcgcag cacgtactcg gatggaagcc ggtcttctcg atcaggatga  
3421 tctggacgaa gagcatcagg ggctcgcgcc agccgaactg ttcgccaggc tcaaggcgag  
3481 catgcccgac ggcgaggatc tcgtcgtgac ccatggcgat gcctgcttgc cgaatatcat  
3541 ggtggaanaa ggccgctttt ctggattcat cgaactgtggc cggctgggtg tggcggaccg  
3601 ctatcaggac atagcgttgg ctaccctgta tattgctgaa gagcttggcg gcgaatgggc  
3661 tgaccgcttc ctcgtgcttt acggtatcgc cgctcccgat tcgcagcgca tcgccttcta  
3721 tcgccttctt gacgagttct tctgagcggg actctggggt tcgaaatgac cgaccaagcg  
3781 acgcccacc tgccatcacg agatttcgat tccaccgccg ctttctatga aaggttgggc  
3841 ttcggaatcg ttttccggga gcccggttg atgatcctcc agcgcgggga tctcatgctg  
3901 gagttcttcg cccaccctag ggggaggcta actgaaacac ggaaggagac aataccgga  
3961 ggaaccgcg ctatgacggc aataaaaaga cagaataaaa cgcacggtgt tgggtcgttt  
4021 gttcataaac gcggggttcg gtcccagggc tggcactctg tcgatacccc accgagacc

```
4081 cattggggcc aatagcccc cgtttcttcc ttttccccac cccaccccc aagttcgggt
4141 gaaggccag ggctcgcagc caacgtcggg gcggcaggcc ctgccatagc ctcaggttac
4201 tcatatatac tttagattga tttaaaactt ctttttaat ttaaaaggat ctaggatgaag
4261 atcctttttg ataattctcat gaccaaaatc ccttaacgtg agttttcgtt ccaactgagcg
4321 tcagaccccc tagaaaagat caaaggatct tcttgagatc ctttttttct gcgcgtaatc
4381 tgctgcttgc aaacaaaaaa accaccgcta ccagcggtagg tttgtttgcc ggatcaagag
4441 ctaccaactc tttttccgaa ggtaactggc ttcagcagag cgcagatacc aaatactggt
4501 cttctagtgt agccgtagtt aggccaccac ttcaagaact ctgtagcacc gcctacatac
4561 ctcgctctgc taatcctggt accagtggct gctgccagtg gcgataagtc gtgtcttacc
4621 gggttggact caagacgata gttaccggat aaggcgcagc ggtcgggctg aacggggggg
4681 tcgtgcacac agcccagctt ggagcgaacg acctacaccg aactgagata cctacagcgt
4741 gagctatgag aaagcggccac gcttcccga gggagaaagg cggacaggta tccggtaagc
4801 ggcagggctg gaacaggaga gcgcacgagg gagcttcag ggggaaacgc ctggtatctt
4861 tatagtcctg tcgggtttcg ccacctctga cttgagcgtc gatTTTTgtg atgctcgtca
4921 ggggggcgga gcctatggaa aaacgccagc aacgcggcct ttttacgggt cctggccttt
4981 tgctggcctt ttgctcacat gttctttcct gcgttatccc ctgattctgt ggataaccgt
5041 attaccgc
```

//

CMV-ST::EGFP,

LOCUS CMV\_ST\_EGFP 4853 bp ds-DNA circular 28-FEB-2020

DEFINITION LAMP1-mGFP Sequencing Result

ORGANISM other sequences; artificial sequences; vectors.

COMMENT LAMP1\_mGFP(34831) from 1 to 6015

COMMENT ApEinfo:methylated:1

FEATURES Location/Qualifiers

misc\_feature 1739..1758

/translation="MAAPGSARRPLLLLLLLLLGLMHCASAAMFMVKNNGTACIMA

NFSAAFSVNYDTKSGPKNMTFDLPSDATVVLNRSSCGKENTSDPSLVIAFGRGHTLTL

NFTRNATRYSVQLMSFVYNLSDTHLFPNASSKEIKTVESITDIRADIDKKYRCVSGTQ

VHMNNVTVLHDATIQAYLSNSSFSRGETRCEQDRPSPTTAPPAPPSPSPSPVPKSPS

```
VDKYNVSGTNGTCLLASMGLQLNLTYERKDNTTVTRLLNINPNKTSASGSCGAHLVTL
ELHSEGTTVLLFQFGMNASSRFFLQGIQLNTILPDARDPAFKAANGSLRALQATVGN
SYKCNAEEHVRVTKAFSVNIFKVVWQAFKVEGGQFGSVEECLLDENSMLIPIAVGGAL
AGLVLIIVLIAYLVGRKRSHAGYQTIGSTGSTGAVDGTAGPGSIATMVSKGEELFT
GVVPILVELDGDVNGHKFSVSGEGEGDATYKGLTLKFICTTGKLPVPWPTLVTTLTYG
VQCFSRYPDHMKQHDFKFSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIEL
KGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQ
QNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMLKEFVTAAGITLGMDELYK*"
    /label=EBV_rev_primer
    /ApEinfo_fwdcolor=#ffcc66
    /ApEinfo_revcolor=#ffcc66
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
    rep_origin    complement(1908..2214)

/translation="MAAPGSARRPLLLLLLLLLGLMHCSAAMFMVKNNGTACIMA
NFSAAFVNYDTKSGPKNMTFDLPSDATVVLNRSSCGKENTSDPSLVIAFGRGHTLTL
NFTRNATRYSVQLMSFVYNLSDTHLFPNASSKEIKTVESITDIRADIDKKYRCVSGTQ
VHMNNVTVLHDATIQAYLSNSSFSRGETRCEQDRPSPPTAPPAPPSPSPSPVPKSPS
VDKYNVSGTNGTCLLASMGLQLNLTYERKDNTTVTRLLNINPNKTSASGSCGAHLVTL
ELHSEGTTVLLFQFGMNASSRFFLQGIQLNTILPDARDPAFKAANGSLRALQATVGN
SYKCNAEEHVRVTKAFSVNIFKVVWQAFKVEGGQFGSVEECLLDENSMLIPIAVGGAL
```

```
AGLVLIVLIAYLVGRKRSHAGYQTIGSTGSGTGAVDGTAGPGSIATMVSKGEELFT
GVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTCLKFICTTGKLPVPWPTLVTTLTYG
VQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIEL
KGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQ
QNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMLKEFVTAAGITLGMDELYK*"
    /label=f1_origin
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#cccccc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
    promoter      2293..2321

/translation="MAAPGSARRPLLLLLLLLLLLGLMHCASAAMFMVKNNGTACIMA
NFSAAFVNYDTKSGPKNMTFDLPSDATVVLNRSSCGKENTSDPSLVIAFGRGHTLTL
NFTRNATRYSVQLMSFVYNLSDTHLFPNASSKEIKTVESITDIRADIDKKYRCVSGTQ
VHMNNVTVLHDATIQAAYLSNSSFSRGETRCEQDRPSPTTAPPAPPSPSPSPVPKSPS
VDKYNVSGTNGTCLLASMGLQLNLTYERKDNTTVTRLLNINPNKTSASGSCGAHLVTL
ELHSEGTTVLLFQFGMNASSRFFLQGIQLNTILPDARDPAFKAANGSLRALQATVGN
SYKCNAEEHVRVTKAFSVNIFKVVWQAFKVEGGQFGSVEECLLDENSMLIPIAVGGAL
AGLVLIVLIAYLVGRKRSHAGYQTIGSTGSGTGAVDGTAGPGSIATMVSKGEELFT
GVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTCLKFICTTGKLPVPWPTLVTTLTYG
VQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIEL
```

```
KGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQ

QNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMLKEFVTAAGITLGMDELYK*"
    /label=AmpR_promoter
    /ApEinfo_fwdcolor=#ccffed
    /ApEinfo_revcolor=#ccffed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
    misc_feature    complement(2387..2407)

/translation="MAAPGSARRPLLLLLLLLLLLGLMHCSAAMFMVKNNGTACIMA

NFSAAFSVNYDTKSGPKNMTFDLPSDATVVLNRSSCGKENTSDPSLVIAFGRGHTLTL

NFTRNATRYSVQLMSFVYNLSDTHLFPNASSKEIKTVESITDIRADIDKKYRCVSGTQ

VHMNNVTVLHDATIQAAYLSNSSFSRGETRCEQDRPSPTTAPPAPPSPSPSPVPKSPS

VDKYNVSGTNGTCLLASMGLQLNLTYERKDNTTVTRLLNINPNKTSASGSCGAHLVTL

ELHSEGTTVLLFQFGMNASSRFFLQGIQLNTILPDARDPAFKAANGSLRALQATVGN

SYKCNAEEHVRVTKAFSVNIFKVVWQAFKVEGGQFGSVEECLLDENSMLIPIAVGGAL

AGLVLIVLIAYLVGRKRSHAGYQTIGSTGSTGSTGAVDGTAGPGSIATMVSKGEELFT

GVVPILVELDGDVNGHKFSVSGEGEDATYGKLTCLKFICTTGKLPVPWPTLVTTLYG

VQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIEL

KGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQ

QNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMLKEFVTAAGITLGMDELYK*"
    /label=pBABE_3_primer
    /ApEinfo_fwdcolor=#ffcc66
    /ApEinfo_revcolor=#ffcc66
```

```

    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    complement(2393..2608)

/translacion="MAAPGSARRPLLLLLLLLLLLGLMHCAAMFMVKNNGTACIMA
NFSAAFVNYDTKSGPKNMTFDLPSDATVVLNRSSCGKENTSDPSLVIAFGRGHTLTL
NFTRNATRYSVQLMSFVYNLSDTHLFPNASSKEIKTVESITDIRADIDKKYRCVSGTQ
VHMNNVTVLHDATIQAYLSNSSFSRGETRCEQDRPSPTTAPPAPPSPSPSPVPKSPS
VDKYNVSGTNGTCLLASMGLQLNLTyerKDNTTVTRLLNINPNKTSASGSCGAHLVTL
ELHSEGTVLLFQFGMNASSRFFLQGIQLNTILPDARDPAFKAANGSLRALQATVGN
SYKCNAEEHVRVTKAFSVNIFKVVWQAFKVEGGQFGSVEECLLDENSMLIPIAVGGAL
AGLVLIVLIAYLVRKRSHAGYQTIGSTGSTGSTGAVDGTAGPGSIATMVSKGEELFT
GVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTlKFICTTGKLPVPWPTLVTTLTYG
VQCFsRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIEL
KGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQ
QNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMLKEFVTAAGITLGMDLYK*"
    /label=SV40_enhancer
    /ApEinfo_fwdcolor=#ffcc66
    /ApEinfo_revcolor=#ffcc66
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
promoter        2405..2673

/translacion="MAAPGSARRPLLLLLLLLLLLGLMHCAAMFMVKNNGTACIMA
```



```
NFSAAFSVNYDTKSGPKNMTFDLPSDATVVLNRSSCGKENTSDPSLVIAFGRGHTLTL
NFTRNATRYSVQLMSFVYNLSDTHLFPNASSKEIKTVESITDIRADIDKKYRCVSGTQ
VHMNNVTVLHDATIQAYLSNSSFSRGETRCEQDRPSPTTAPPAPPSPSPSPVPKSPS
VDKYNVSGTNGTCLLASMGLQLNLTYERKDNTTVTRLLNINPNKTSASGSCGAHLVTL
ELHSEGTTVLLFQFGMNASSRFFLQGIQLNTILPDARDPAFKAANGSLRALQATVGN
SYKCNAEEHVRVTKAFSVNIFKVVWQAFKVEGGQFGSVEECLLDENSMLIPIAVGGAL
AGLVLIVLIAAYLVGRKRSHAGYQTIGSTGSTGSTGAVDGTAGPGSIATMVSKGEELFT
GVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTCLKFICTTGKLPVPWPTLVTTLTYG
VQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIEL
KGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQ
QNTPIGDGPVLLPDNYLSTQSALSKDPNEKRDHMLKEFVTAAGITLGMDELYK*"
    /label=SV40_promoter
    /ApEinfo_fwdcolor=#ccffed
    /ApEinfo_revcolor=#ccffed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
    rep_origin    2572..2649

/translation="MAAPGSARRPLLLLLLLLLLLGLMHCASAAMFMVKNNGTACIMA
NFSAAFSVNYDTKSGPKNMTFDLPSDATVVLNRSSCGKENTSDPSLVIAFGRGHTLTL
NFTRNATRYSVQLMSFVYNLSDTHLFPNASSKEIKTVESITDIRADIDKKYRCVSGTQ
VHMNNVTVLHDATIQAYLSNSSFSRGETRCEQDRPSPTTAPPAPPSPSPSPVPKSPS
```

```
VDKYNVSGTNGTCLLASMGLQLNLTYERKDNTTVTRLLNINPNKTSASGSCGAHLVTL
ELHSEGTTVLLFQFGMNASSRFFLQGIQLNTILPDARDPAFKAANGSLRALQATVGN
SYKCNAEEHVRVTKAFSVNIFKVVWQAFKVEGGQFGSVEECLLDENSMLIPIAVGGAL
AGLVLIIVLIAYLVGRKRSHAGYQTIGSTGSTGSTGAVDGTAGPGSIATMVSKGEELFT
GVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTCLKFICTTGKLPVPWPTLVTTLTYG
VQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIEL
KGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQ
QNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMLKEFVTAAGITLGMDELYK*"
    /label=SV40_origin
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    2634..2653

/translation="MAAPGSARRPLLLLLLLLLLLGLMHCASAAMFMVKNNGTACIMA
NFSAAFVNYDTKSGPKNMTFDLPSDATVVLNRSSCGKENTSDPSLVIAFGRGHTLTL
NFTRNATRYSVQLMSFVYNLSDTHLFPNASSKEIKTVESITDIRADIDKKYRCVSGTQ
VHMNNVTVLHDATIQAYLSNSSFSRGETRCEQDRPSPPTAPPAPPSPSPSPVPKSPS
VDKYNVSGTNGTCLLASMGLQLNLTYERKDNTTVTRLLNINPNKTSASGSCGAHLVTL
ELHSEGTTVLLFQFGMNASSRFFLQGIQLNTILPDARDPAFKAANGSLRALQATVGN
SYKCNAEEHVRVTKAFSVNIFKVVWQAFKVEGGQFGSVEECLLDENSMLIPIAVGGAL
```

```
AGLVLIVLIAYLVGRKRSHAGYQTIGSTGSGTGAVDGTAGPGSIATMVSKEELFT
GVVPILVELDGDVNGHKFSVSGEGEDATYGKLTCLKFICTTGKLPVPWPTLVTTLYG
VQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIEL
KGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQ
QNTPIGDGPVLLPDNYLSTQSALSKDPNEKRDHMLKEFVTAAGITLGMDELYK*"
    /label=SV40pro_F_primer
    /ApEinfo_fwdcolor=#ffcc66
    /ApEinfo_revcolor=#ffcc66
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
    CDS      2756..3550

/translation="MIEQDGLHAGSPAAWVERLFGYDWAQQTIGCSDAAVFRLSAQGR
PVLFVKTDLSGALNELQDEAARLSWLATTGVPCAAVLDVVTEAGRDWLLLGEVPGQDL
LSSHLAPAEEKVSIMADAMRRLLHTLDPATCPFDHQAKHRIERARTRMEAGLVDQDDLDE
EHQGLAPAELEFARLKASMPDGEDLVVTHGDACLPNIMVENGRFSGFIDCGRLGVADRY
    QDIALATRDIAEELGGEWADRFLVLYGIAAPDSQRIAFYRLLDEFF*"
    /label=ORF frame 3
    /ApEinfo_fwdcolor=#99ccff
    /ApEinfo_revcolor=#99ccff
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
    gene      2759..3547
    /gene="NeoR/KanR"

/translation="MIEQDGLHAGSPAAWVERLFGYDWAQQTIGCSDAAVFRLSAQGR
PVLFVKTDLSGALNELQDEAARLSWLATTGVPCAAVLDVVTEAGRDWLLLGEVPGQDL
```

```
LSSHLAPAEKVSIMADAMRRLHTLDPATCPFDHQAKHRIERARTRMEAGLVDQDDLDE

EHQGLAPAEELFARLKASMPDGEDLVVTHGDACLPNIMVENGRFSGFIDCGRLGVADRY
    QDIALATRDIAEELGGEWADRFLVLYGIAAPDSQRIAFYRLLDEFF*"
    /label=NeoR/KanR
    /ApEinfo_fwdcolor=#ffffcc
    /ApEinfo_revcolor=#ffffcc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
CDS        complement(3065..3601)

/translation="MAGWASLGRSFRTPESEELVKKAIEGDALRIGSGDTVKHEEA

VSPFAAKLFSNITGSQRYVLI AVRHTQPATVDESRAAIFHHDIRQAGIAMGHDEILA

VGHARLEPGEQFGWREPLMLFVQIILIDKTGFHPSTCSLDAMFRLVVEWAGSRIKRMQ
    PPHCISHDGYFLGRSKVR*"
    /label=ORF frame 2
    /ApEinfo_fwdcolor=#99ccff
    /ApEinfo_revcolor=#99ccff
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    635..766
    /label=ST
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
terminator    3725..3994

/translation="MAGWASLGRSFRTPESEELVKKAIEGDALRIGSGDTVKHEEA

VSPFAAKLFSNITGSQRYVLI AVRHTQPATVDESRAAIFHHDIRQAGIAMGHDEILA

VGHARLEPGEQFGWREPLMLFVQIILIDKTGFHPSTCSLDAMFRLVVEWAGSRIKRMQ
    PPHCISHDGYFLGRSKVR*"

```

```

        /label=TK_PA_terminator
        /ApEinfo_fwdcolor=pink
        /ApEinfo_revcolor=pink
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
rep_origin      4142..4761

/translation="MAGWASLGRSFRTPEsrSEELVKKaIEGDALRIGSGDtvKHEEA
VSPFAAKLFSNITGSQRYVLIaVRHTQPATVDESrKAAIFHHDIRQAGIAMGHDEILA
VGHARLEPGEQFGWREPLMLFVQIILIDKTGFHPSTCSLDAMFRLVVEWAGSRIKRMQ
PPHCISHDGYFLGRSKVR*"
        /label=pBR322_origin
        /ApEinfo_fwdcolor=cyan
        /ApEinfo_revcolor=#00ff00
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
promoter        17..569
        /label=CMV_imnearlY_promoter
        /ApEinfo_fwdcolor=#ccffed
        /ApEinfo_revcolor=#ccffed
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
promoter        527..596
        /label=CMV_promoter
        /ApEinfo_fwdcolor=#ccffed
        /ApEinfo_revcolor=#ccffed
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
misc_feature    526..546
        /label=CMV_fwd_primer
        /ApEinfo_fwdcolor=#ffcc66
        /ApEinfo_revcolor=#ffcc66
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
```

```
gene      809..1522
          /gene="EGFP"

/translacion="MVSKGEELFTGVVPIVELDGDVNGHKFSVSGEGEGDATYGKLT
LKFICTTGKLPVPWPTLVTTLTYGVCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFK
DDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNNSHNVYIMADKQKN
GIKVNFKIRHNIEDGSVQLADHYQNTPIGDGPVLLPDNHYLSTQSALS KDPNEKRDH
          MVLLEFVTAAGITLGMDELYKSGLRSAQASNSAVDGTAGPGSTGSR*"
          /label=EGFP
          /ApEinfo_fwdcolor=#66ff66
          /ApEinfo_revcolor=#ffffcc
          /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
          width 5 offset 0
misc_feature complement(851..872)

/translacion="MVSKGEELFTGVVPIVELDGDVNGHKFSVSGEGEGDATYGKLT
LKFICTTGKLPVPWPTLVTTLTYGVCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFK
DDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNNSHNVYIMADKQKN
GIKVNFKIRHNIEDGSVQLADHYQNTPIGDGPVLLPDNHYLSTQSALS KDPNEKRDH
          MVLLEFVTAAGITLGMDELYKSGLRSAQASNSAVDGTAGPGSTGSR*"
          /label=EGFP_N_primer
          /ApEinfo_fwdcolor=#ffcc66
          /ApEinfo_revcolor=#ffcc66
          /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
          width 5 offset 0
misc_feature      989..1018

/translacion="MVSKGEELFTGVVPIVELDGDVNGHKFSVSGEGEGDATYGKLT
LKFICTTGKLPVPWPTLVTTLTYGVCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFK
```

```
DDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNNSHNVYIMADKQKN

GIKVNFKIRHNIEDGSVQLADHYQNTPIGDGPVLLPDNHYLSTQSALS KDPNEKRDH
    MVLLEFVTAAGITLGMDELYKSGLR SRAQASNSAVDGTAGPGSTGSR*"
    /label=Y66 (EGFP)
    /ApEinfo_fwdcolor=#ffcc66
    /ApEinfo_revcolor=#ffcc66
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    1459..1480

/translation="MVSKGEELFTGVVPIVELDGDVNGHKFSVSGEGEGDATYGKLT

LKFICTTGKLPVPWPTLVTTLTYGVCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFK

DDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNNSHNVYIMADKQKN

GIKVNFKIRHNIEDGSVQLADHYQNTPIGDGPVLLPDNHYLSTQSALS KDPNEKRDH
    MVLLEFVTAAGITLGMDELYKSGLR SRAQASNSAVDGTAGPGSTGSR*"
    /label=EGFP_C_primer
    /ApEinfo_fwdcolor=#ffcc66
    /ApEinfo_revcolor=#ffcc66
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0

ORIGIN
    1 catgcattag ttattaatag taatcaatta cggggtcatt agttcatagc ccatatatgg
    61 agttccgcgt tacataactt acggtaaatg gcccgccctgg ctgaccgcc aacgaccccc
    121 gccattgac gtcaataatg acgtatgttc ccatagtaac gccaataggg actttccatt
    181 gacgtcaatg ggtggagtat ttacggtaaa ctgccactt ggcagtacat caagtgtatc
    241 atatgccaag tacgccccct attgacgtca atgacggtaa atggcccgcc tggcattatg
    301 cccagtacat gaccttatgg gactttccta cttggcagta catctacgta ttagtcatcg
    361 ctattaccat ggtgatgcgg ttttggcagt acatcaatgg gcgtggatag cggtttgact
    421 cacggggatt tccaagtctc caccattg acgtcaatgg gagtttgttt tggcaccaaa
    481 atcaacggga ctttcaaaa tgtcgtaca actccgccc attgacgcaa atgggcggta
    541 ggcgtgtacg gtgggaggtc tatataagca gagctggtt agtgaaccgt cagatccgct
```

601 agcgctaccG GACTCAGATC TCGAGctcgc caccATGATT CACACCAACC TGAAGAAAA  
661 GTTCAGCTGC TGCCTCTGG TCTTTCTTCT GTTTGCAGTC ATCTGTGTGT GGAAGGAAAA  
721 GAAGAAAGGG AGTTACTATG ATTCCTTTAA ATTGCAAACC AAGGGGTCGA CGGTACCGCG  
781 GGCCCGGGAT CCACCGGTcg ccaccATGGT GAGCAAGGGC GAGGAGCTGT TCACCGGGGT  
841 GGTGCCCATC CTGGTCGAGC TGGACGGCGA CGTAAACGGC CACAAGTTCA GCGTGTCCGG  
901 CGAGGGCGAG GGCATGCCA CCTACGGCAA GCTGACCCTG AAGTTCATCT GCACCACCGG  
961 CAAGCTGCCC GTGCCCTGGC CCACCCTCGT GACCACCCTG ACCTACGGCG TGCAGTGCTT  
1021 CAGCCGCTAC CCCGACCACA TGAAGCAGCA CGACTTCTTC AAGTCCGCCA TGCCCGAAGG  
1081 CTACGTCCAG GAGCGCACCA TCTTCTTCAA GGACGACGGC AACTACAAGA CCCGCGCCGA  
1141 GGTGAAGTTC GAGGGCGACA CCCTGGTGAA CCGCATCGAG CTGAAGGGCA TCGACTTCAA  
1201 GGAGGACGGC AACATCCTGG GGCACAAGCT GGAGTACAAC TACAACAGCC ACAACGTCTA  
1261 TATCATGGCC GACAAGCAGA AGAACGGCAT CAAGGTGAAC TTCAAGATCC GCCACAACAT  
1321 CGAGGACGGC AGCGTGCAGC TCGCCGACCA CTACCAGCAG AACACCCCA TCGGCGACGG  
1381 CCCCCTGCTG CTGCCCGACA ACCACTACCT GAGCACCCAG TCCGCCCTGA GCAAAGACCC  
1441 CAACGAGAAG CGCGATCACA TGGTCCTGCT GGAGTTCGTG ACCGCCGCCG GGATCACTCT  
1501 CGGCATGGAC GAGCTGTACA AGTAAAGCGG CCGCGACTCT AGATCATAAT CAGCCATACC  
1561 ACATTTGTAG AGGTTTTACT TGCTTTAAAA AACCTCCAC ACCTCCCCCT GAACCTGAAA  
1621 CATAAAATGA ATGCAATTGT TGTTGTTAAC TTGTTTATTG CAGTTATAA TGGTTACAAA  
1681 TAAAGCAATA GCATCACAAA TTTCAAAAT AAAGCATTTT TTTCACTGCA TTCTAGTTGT  
1741 GTTTTGTCCA AACTCATCAA TGTATCTTAA GGCGTAAATT GTAAGCGTTA ATATTTTGT  
1801 AAAATTCGCG TTAAATTTTT GTTAAATCAG CTCATTTTTT AACCAATAGG CCGAAATCGG  
1861 CAAAATCCCT TATAAATCAA AAGAATAGAC CGAGATAGGG TTGAGTGTG TTCCAGTTTG  
1921 GAACAAGAGT CCACTATTAA AGAACGTGGA CTCCAACGTC AAAGGGCGAA AAACCGTCTA  
1981 TCAGGGCGAT GGCCCACTAC GTGAACCATC ACCCTAATCA AGTTTTTTGG GGTCGAGGTG  
2041 CCGTAAAGCA CTAAATCGGA ACCCTAAAGG GAGCCCCGA TTTAGAGCTT GACGGGGAAA  
2101 GCCGGCGAAC GTGGCGAGAA AGGAAGGGAA GAAAGCGAAA GGAGCGGGCG CTAGGGCGCT  
2161 GGCAAGTGTA GCGGTCACGC TGCGCGTAAC CACCACACCC GCCGCGCTTA ATGCGCCGCT  
2221 ACAGGGCGCG TCAGGTGGCA CTTTTCGGGG AAATGTGCGC GGAACCCCTA TTTGTTTATT  
2281 TTTCTAAATA CATTCAAATA TGTATCCGCT CATGAGACAA TAACCCTGAT AAATGCTTCA  
2341 ATAATATTGA AAAAGGAAGA GTCCTGAGGC GGAAAGAACC AGCTGTGGAA TGTGTGTCAG  
2401 TTAGGGTGTG GAAAGTCCCC AGGCTCCCCA GCAGGCAGAA GTATGCAAAG CATGCATCTC  
2461 AATTAGTCAG CAACCAGGTG TGGAAAGTCC CCAGGCTCCC CAGCAGGCAG AAGTATGCAA  
2521 AGCATGCATC TCAATTAGTC AGCAACCATA GTCCC GCCC TAACTCCGCC CATCCCGCCC  
2581 CTAACTCCGC CCAGTTCCGC CCATTCTCGC CCCCATGGCT GACTAATTTT TTTTATTTAT  
2641 GCAGAGGCCG AGGCCGCCTC GGCCTCTGAG CTATTCCAGA AGTAGTGAGG AGGCTTTTTT  
2701 GGAGGCCTAG GCTTTTGCAA AGATCGATCA AGAGACAGGA TGAGGATCGT TTCGCATGAT



2761 TGAACAAGAT GGATTGCACG CAGGTTCTCC GGCCGCTTGG GTGGAGAGGC TATTCCGGCTA  
2821 TGACTGGGCA CAACAGACAA TCGGCTGCTC TGATGCCGCC GTGTTCCGGC TGTCAGCGCA  
2881 GGGGCGCCCG GTTCTTTTTG TCAAGACCGA CCTGTCCGGT GCCCTGAATG AACTGCAAGA  
2941 CGAGGCAGCG CGGCTATCGT GGCTGGCCAC GACGGGCGTT CTTGCGCAG CTGTGCTCGA  
3001 CGTTGTCACT GAAGCGGGAA GGGACTGGCT GCTATTGGGC GAAGTGCCGG GGCAGGATCT  
3061 CCTGTCATCT CACCTTGCTC CTGCCGAGAA AGTATCCATC ATGGCTGATG CAATGCGGCG  
3121 GCTGCATACG CTTGATCCGG CTACCTGCC ATTCGACCAC CAAGCGAAAC ATCGCATCGA  
3181 GCGAGCACGT ACTCGGATGG AAGCCGGTCT TGTCGATCAG GATGATCTGG ACGAAGAGCA  
3241 TCAGGGGCTC GCGCCAGCCG AACTGTTCGC CAGGCTCAAG GCGAGCATGC CCGACGGCGA  
3301 GGATCTCGTC GTGACCCATG GCGATGCCTG CTTGCCGAAT ATCATGGTGG AAAATGGCCG  
3361 CTTTTCTGGA TTCATCGACT GTGGCCGGCT GGGTGTGGCG GACCGCTATC AGGACATAGC  
3421 GTTGGCTACC CGTGATATTG CTGAAGAGCT TGGCGGCGAA TGGGCTGACC GCTTCCTCGT  
3481 GCTTTACGGT ATCGCCGCTC CCGATTGCGA GCGCATCGCC TTCTATCGCC TTCTTGACGA  
3541 GTTCTTCTGA GCGGGACTCT GGGGTTGCAA ATGACCGACC AAGCGACGCC CAACCTGCCA  
3601 TCACGAGATT TCGATTCCAC CGCCGCCTTC TATGAAAGGT TGGGCTTCGG AATCGTTTTC  
3661 CGGGACGCCG GCTGGATGAT CCTCCAGCGC GGGGATCTCA TGCTGGAGTT CTTGCCCCAC  
3721 CCTAGGGGGA GGCTAACTGA AACACGGAAG GAGACAATAC CGGAAGGAAC CCGCGCTATG  
3781 ACGGCAATAA AAAGACAGAA TAAAACGCAC GGTGTTGGGT CGTTTGTTC A TAAACGCGGG  
3841 GTTCGGTCCC AGGGCTGGCA CTCTGTCGAT ACCCCACCGA GACCCCAT TG GGGCCAATAC  
3901 GCCCGCGTTT CTTCTTTTT CCCCACCCAC CCCCAGTT CGGGTGAAGG CCCAGGGCTC  
3961 GCAGCCAACG TCGGGGCGGC AGGCCCTGCC ATAGCCTCAG GTTACTCATA TATACTTTAG  
4021 ATTGATTTAA AACTTCATTT TTAATTTAAA AGGATCTAGG TGAAGATCCT TTTTGATAAT  
4081 CTCATGACCA AAATCCCTTA ACGTGAGTTT TCGTTCCACT GAGCGTCAGA CCCCCTAGAA  
4141 AAGATCAAAG GATCTTCTTG AGATCTTTT TTTCTGCGCG TAATCTGCTG CTTGCAAACA  
4201 AAAAAACCAC CGTACCAGC GGTGTTTGT TTGCCGGATC AAGAGCTACC AACTCTTTTT  
4261 CCGAAGGTAA CTGGCTTCAG CAGAGCGCAG ATACCAAATA CTGTTCTTCT AGTGTAGCCG  
4321 TAGTTAGGCC ACCACTTCAA GAACTCTGTA GCACCGCCTA CATACTCGC TCTGCTAATC  
4381 CTGTTACCAG TGGCTGCTGC CAGTGGCGAT AAGTCGTGTC TTACCGGGTT GGA CTCAAGA  
4441 CGATAGTTAC CGGATAAGGC GCAGCGGTCG GGCTGAACGG GGGGTTCTGT CACACAGCCC  
4501 AGCTTGAGC GAACGACCTA CACCGAAGT AGATACCTAC AGCGTGAGCT ATGAGAAAGC  
4561 GCCACGCTTC CCGAAGGGAG AAAGGCGGAC AGGTATCCGG TAAGCGGCAG GGTCGGAACA  
4621 GGAGAGCGCA CGAGGGAGCT TCCAGGGGGA AACGCCTGGT ATCTTTATAG TCCTGTGGG  
4681 TTTCCGCCACC TCTGACTTGA GCGTCGATTT TTGTGATGCT CGTCAGGGGG GCGGAGCCTA  
4741 TGGAAAAACG CCAGCAACGC GGCCTTTTTA CGGTTCTGG CTTTTGCTG GCCTTTTGCT  
4801 CACATGTTCT TTCCTGCGTT ATCCCCTGAT TCTGTGGATA ACCGTATTAC CGC

//

pMT-GalT::EGFP					
LOCUS	pMT_GalT_EGFP	5661 bp	ds-DNA	circular	28-FEB-2020
DEFINITION	pMT-puro Sequencing Result				
ORGANISM	other sequences; artificial sequences; vectors.				
COMMENT	pMT-puro from 1 to 4724				
COMMENT	ApEinfo:methylated:1				
FEATURES	Location/Qualifiers				
misc_feature	4464..4640				
	/label=GalT				
	/ApEinfo_fwdcolor=cyan				
	/ApEinfo_revcolor=#00ff00				
	/ApEinfo_graphicformat=arrow_data	{0 1 2 0 0 -1} {} 0}			
	width 5 offset 0				
gene	4689..5402				
	/gene="EGFP"				
/translation="MVSKGEELFTGVVPIVELDGDVNGHKFSVSGEGEGDATYGKLT					
LKFICTTGKLPVPWPTLVTTLTYGVCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFK					
DDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNNSHNVYIMADKQKN					
GIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDH					
	MVLLFVTAAGITLGMDELYKSGLRSAQASNSAVDGTAGPGSTGSR**				
	/label=EGFP				
	/ApEinfo_fwdcolor=#66ff66				
	/ApEinfo_revcolor=#ffffcc				
	/ApEinfo_graphicformat=arrow_data	{0 1 2 0 0 -1} {} 0}			
	width 5 offset 0				
misc_feature	complement(4731..4752)				
/translation="MVSKGEELFTGVVPIVELDGDVNGHKFSVSGEGEGDATYGKLT					
LKFICTTGKLPVPWPTLVTTLTYGVCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFK					

```
DDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNNSHNVYIMADKQKN

GIKVNFKIRHNIEDGSVQLADHYQNTPIGDGPVLLPDNHYLSTQSALS KDPNEKRDH
    MVLLEFVTAAGITLGMDELYKSGLR SRAQASNSAVDGTAGPGSTGSR*"
    /label=EGFP_N_primer
    /ApEinfo_fwdcolor=#ffcc66
    /ApEinfo_revcolor=#ffcc66
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
    misc_feature    4869..4898

/translation="MVSKGEELFTGVVPIVELDGDVNGHKFSVSGEGEGDATYGKLT

LKFICTTGKLPVPWPTLVTTLTYGVCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFK

DDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNNSHNVYIMADKQKN

GIKVNFKIRHNIEDGSVQLADHYQNTPIGDGPVLLPDNHYLSTQSALS KDPNEKRDH
    MVLLEFVTAAGITLGMDELYKSGLR SRAQASNSAVDGTAGPGSTGSR*"
    /label=Y66 (EGFP)
    /ApEinfo_fwdcolor=#ffcc66
    /ApEinfo_revcolor=#ffcc66
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
    misc_feature    5339..5360

/translation="MVSKGEELFTGVVPIVELDGDVNGHKFSVSGEGEGDATYGKLT

LKFICTTGKLPVPWPTLVTTLTYGVCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFK

DDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNNSHNVYIMADKQKN

GIKVNFKIRHNIEDGSVQLADHYQNTPIGDGPVLLPDNHYLSTQSALS KDPNEKRDH
    MVLLEFVTAAGITLGMDELYKSGLR SRAQASNSAVDGTAGPGSTGSR*"
    /label=EGFP_C_primer
    /ApEinfo_fwdcolor=#ffcc66
```

```

    /ApEinfo_revcolor=#ffcc66
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature    complement(3606..3628)
    /label=pGEX_3_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
promoter        complement(3419..3447)
    /label=AmpR_promoter
    /ApEinfo_fwdcolor=#ccffed
    /ApEinfo_revcolor=#ccffed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
gene            complement(2517..3377)
    /gene="Ampicillin"
    /label=Ampicillin
    /ApEinfo_fwdcolor=#ffffcc
    /ApEinfo_revcolor=#ffffcc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
rep_origin      complement(1743..2362)

/translation="MSIQHFRVALIPFFAAFLPVFAHPETLVKVKDAEDQLGARVGY
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHVTRL
DRWEPELNEAIPNDERDTTMPVAMATTLRKLTLGELLTLASRQQLIDWMEADKVAGPL
LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
    EIGASLIKHW*"
    /label=pBR322_origin
    /ApEinfo_fwdcolor=pink
```

```

    /ApEinfo_revcolor=pink
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
promoter    complement(1405..1434)

/translation="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGY
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLTTIGGPKELTAFLHNMGDHVTRL
DRWEPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPL
LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
    EIGASLIKHW*"
    /label=lac_promoter
    /ApEinfo_fwdcolor=#ccffed
    /ApEinfo_revcolor=#ccffed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    complement(1369..1391)

/translation="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGY
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLTTIGGPKELTAFLHNMGDHVTRL
DRWEPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPL
LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
    EIGASLIKHW*"
    /label=M13_pUC_rev_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}

```

```
width 5 offset 0
promoter complement(1352..1370)

/translation="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGY
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHVTRL
DRWEPELNEAIPNDERDTTMPVAMATTLRKLTTGELLTLASRQQLIDWMEADKVAGPL
LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
EIGASLIKHW*"
/label=M13_reverse_primer
/ApEinfo_fwdcolor=#ccffed
/ApEinfo_revcolor=#ccffed
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind complement(1352..1372)
/label=M13-rev
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=green
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature complement(1574..1577)
/label=Sac1 GAGCT/C (1/2)
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
rep_origin complement(1740..2422)
/label=ColE1 origin
/ApEinfo_fwdcolor=gray50
/ApEinfo_revcolor=gray50
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
```

```
misc_recomb    1574..1580
                /label=BstQI GCTCTTCN/NNN
                /ApEinfo_fwdcolor=#ffb500
                /ApEinfo_revcolor=#ff7e07
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
misc_binding    complement(1378..1400)
                /label=Lac0
                /ApEinfo_fwdcolor=#6495ed
                /ApEinfo_revcolor=#6495ed
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
CDS             complement(2520..3179)
                /label=AmpR
                /ApEinfo_fwdcolor=yellow
                /ApEinfo_revcolor=yellow
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
primer_bind     complement(1352..1372)
                /label=M13R_GW
                /ApEinfo_fwdcolor=#ff00bd
                /ApEinfo_revcolor=#ff0003
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
primer_bind     3491..3508
                /label=pQE60-F
                /ApEinfo_fwdcolor=#ff00bd
                /ApEinfo_revcolor=#ff0003
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
gene           complement(143..742)
                /gene="puro (variant)"

/translation="MGSCAPFGRALRVVGRASGTGLAGHAPGARSFGHLDVGGDGAE
PLVEGEVAGRGLQEGGHPGALGRLHSGEHDGAAQTLALVVGRDADGGQEPRGLLGPV
```

RRQEAFHLLLRGQPGTAQLGHARADLGEHRPRFDALRRGPDRHRGAVVRDPHLADVEP

DAREEEFLQLGDPLDVAVRVDGVARGGVVGERGGEGAYGPGDVVAGGEAHRGLVLGHG

RSSPCEGSGAWVRGWRHRSWRPTCRHASYPARAARGICCNL\*"

/label=puro (variant)

/ApEinfo\_fwdcolor=#ffffcc

/ApEinfo\_revcolor=#ffffcc

/ApEinfo\_graphicformat=arrow\_data {{0 1 2 0 0 -1}} {} 0}

width 5 offset 0

misc\_feature complement(843..1201)

/label=Copia Promoter?

/ApEinfo\_fwdcolor=#cde7f7

/ApEinfo\_revcolor=#cde7f7

/ApEinfo\_graphicformat=arrow\_data {{0 1 2 0 0 -1}} {} 0}

width 5 offset 0

misc\_feature complement(3820..3975)

/label=lacZ\_a

/ApEinfo\_fwdcolor=#ff3600

/ApEinfo\_revcolor=#ff3600

/ApEinfo\_graphicformat=arrow\_data {{0 1 2 0 0 -1}} {} 0}

width 5 offset 0

promoter 3956..3972

/label=M13\_forward20\_primer

/ApEinfo\_fwdcolor=#ccffed

/ApEinfo\_revcolor=#ccffed

/ApEinfo\_graphicformat=arrow\_data {{0 1 2 0 0 -1}} {} 0}

width 5 offset 0

misc\_feature 3941..3963

/label=M13\_pUC\_fwd\_primer

/ApEinfo\_fwdcolor=#ff3600

/ApEinfo\_revcolor=#ff3600

/ApEinfo\_graphicformat=arrow\_data {{0 1 2 0 0 -1}} {} 0}

width 5 offset 0

misc\_feature 3982..4349

/label=MT-promoter



```
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    3955..3972
    /label=M13-fwd
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=green
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
CDS
    complement(3816..3884)
    /label=LacZ alpha
    /ApEinfo_fwdcolor=#6495ed
    /ApEinfo_revcolor=#6495ed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    3929..3952
    /label=M13F
    /ApEinfo_fwdcolor=#0a00ff
    /ApEinfo_revcolor=#0a00ff
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    3955..3972
    /label=M13F_GW
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature   4385..4402
    /label=Metallothionein_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature   complement(5572..5591)
    /label=EBV_rev_primer
```

```
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    complement(5529..5546)
    /label=BGH_rev_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    5489..5506
    /label=6xHis
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    5438..5479
    /label=V5
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    5414..5419
    /label=XhoI
    /ApEinfo_fwdcolor=#ffcc66
    /ApEinfo_revcolor=#ffcc66
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
```

ORIGIN

```
1 ataagctgca ataacaaagt tctagagtcg gtgggcctcg ggggcgggtg cggggtcggc
61 ggggccgcc cgggtggctt cggtcggagc catggggtcg tgcgctcctt tcggtcgggc
121 gctgcgggtc gtggggcggg cgtcaggcac cgggcttgcg ggtcatgcac caggtgcgcg
181 gtccttcggg cacctcgacg tcggcgggta cgggtaagcc gagccgctcg tagaagggga
241 ggttgcgggg cgcgagggtc tccaggaagg cgggcacccc ggcgcgctcg gccgcctcca
301 ctccggggag cagcagggcg ctgcccagac cttgcccctg gtggtcgggc gagacgccga
361 cgggtggccag gaaccacgcg ggctccttgg gccggtgcgg cgccaggagg ctttccatct
```

421 gttgctgcgc ggccagccgg gaaccgctca actcggccat gcgcgggccc atctcggcga  
481 acaccgcccc cgcttcgacg ctctccggcg tggteccagac cgccaccgcg gcgcccgcgt  
541 ccgcgaccca caccttgccg atgtcgagcc cgacgcgcgt gaggaagagt tcttcgagct  
601 cggtgaccgg ctcgatgtgg cggteccgggt cgacgggtgtg gcgcgtggcg gggtagtcgg  
661 cgaacgcggc ggcgaggggtg cgtacggccc gggggacgtc gtcgcgggtg gcgaggcgca  
721 ccgtgggctt gtactcggtc atggaaggtc gtctccttgt gaggggtcag gggcgtgggt  
781 caggggatgg tggcggcacc ggtcgtggcg gccgacctg aggcatgcaa gctatcgaat  
841 tcctgcagcc cgggggatct gttgtaattt ataatttata tttcctttct taataaataa  
901 ataaatagtc aagtttatgt ttgagtttta tgatttata ttttaagttat ttcaactgca  
961 acaccagcac cagcactac ttacagcaaa aaacgtacaa gaaggaaaga aggaataaaa  
1021 agagtggat tctcttacia tatgttttat ggcataaaag gtgtggccat tcatatcaaa  
1081 tataaagtag tgttgtttaa cgttactttt gtaggttgaa tagtatattc caacagatga  
1141 tgaggggttc ccaatcctaa acccatttgc cgttcccaga agcatgaaac caccacgcac  
1201 cggatcctct agaacaacia caattgcatt cattttatgt ttcaggttca gggggaggtg  
1261 tgggaggttt tttaaagcaa gtaaacctc taaaaatgtg gtatggctga ttatgatcag  
1321 tcgacctgca ggcattgAAG CTTggcgtaa tcatggctat agctgtttcc tgtgtgaaat  
1381 tgttatccgc tcacaattcc acacaacata cgagccggaa gcataaagt taaagcctgg  
1441 ggtgcctaag gagtgagcta actcacatta attgcgttgc gtcactgcc cgctttccag  
1501 tcgggaaacc tgcctgcca gctgcattaa tgaatcggc aacgcgcggg gagaggcgg  
1561 ttgcgtattg ggcGCTCTC cgcttcctcg ctactgact cgctgcgctc ggtcgttcgg  
1621 ctgcggcgag cggtatcagc tcaactcaag gcggtatac ggttatccac agaatcaggg  
1681 gataacgcag gaaagaacat gtgagcaaaa ggccagcaaa aggccaggaa ccgtaaaaag  
1741 gccgcgttgc tggcgttttt ccataggctc cgccccctg acgagcatca caaaaatcga  
1801 cgctcaagtc agaggtggcg aaacccgaca ggactataaa gataccaggc gtttccccct  
1861 ggaagctccc tcgtgcgctc tcctgttccg accctgccg ttaccggata cctgtccgcc  
1921 tttctccctt cgggaagcgt ggcgctttct catagctcac gctgtaggta tctcagttcg  
1981 gtgtaggctg ttcgctcaa gctgggctgt gtgcacgaac cccccgtta gcccgaccgc  
2041 tgcgccttat ccggtacta tcgtcttgag tccaaccgg taagacacga cttatcgcca  
2101 ctggcagcag ccaactgtaa caggattagc agagcgagg atgtaggcgg tgctacagag  
2161 ttcttgaagt ggtggcctaa ctacggctac actagaagaa cagtatttgg tatctgcgct  
2221 ctgctgaagc cagttacctt cggaaaaaga gttggtagct cttgatccgg caaacaacc  
2281 accgctggta gcggtgggtt ttttggttgc aagcagcaga ttacgcgcag aaaaaagga  
2341 tctcaagaag atcctttgat ctttctacg gggctgacg ctacgtggaa cgaaaactca  
2401 cgtaagggg ttttggtcat gagattatca aaaaggatct tcacctagat ctttttaaat  
2461 taaaaatgaa gttttaaatc aatctaaagt atatatgagt aaacttggtc tgacagttac  
2521 caatgcttaa tcagtgggc acctatctca gcgatctgtc tatttcgttc atccatagtt

```
2581 gcctgactcc ccgtcgtgta gataactacg atacgggagg gcttaccatc tggccccagt
2641 gctgcaatga taccgcgaga cccacgetca ccggetccag atttatcagc aataaaccag
2701 ccagccggaa gggccgagcg cagaagtggc cctgcaactt tatccgcctc catccagtct
2761 attaatgtt gccgggaagc tagagtaagt agttcgccag ttaatagttt gcgcaacggt
2821 gttgccattg ctacaggcat cgtgggtgca cgctcgtcgt ttggtatggc ttcattcagc
2881 tccggttccc aacgatcaag gcgagttaca tgatcccca tgttgtgcaa aaaagcgggt
2941 agctccttcg gtcctccgat cgttgtcaga agtaagtgg ccgcagtgtt atcactcatg
3001 gttatggcag cactgcataa ttctcttact gtcatgcat ccgtaagatg cttttctgtg
3061 actggtgagt actcaaccaa gtcattctga gaatagtgtg tgcggcgacc gagttgctct
3121 tgccccgcgt caatacggga taataccgcg ccacatagca gaactttaa agtgctcatc
3181 attggaanaac gttcttcggg gcgaaaactc tcaaggatct taccgctgtt gagatccagt
3241 tcgatgtaac ccaactcgtc acccaactga tcttcagcat cttttacttt caccagcgtt
3301 tctgggtgag caaaaacagg aaggcaaat gccgcaaaa agggaataag ggcgacacgg
3361 aatgttgaa tactcatact ctctctttt caatattatt gaagcattta tcagggttat
3421 tgtctcatga gcggatacat atttgaatgt atttagaaaa ataaacaaat aggggttccg
3481 gcacacattc cccgaaaagt gccacctgac gtctaagaaa ccattattat catgacatta
3541 acctataaaa ataggcgtat cacgaggccc tttcgtctcg cgcgtttcgg tgatgacggt
3601 gaaaacctct gacacatgca gctcccggag acggtcacag cttgtctgta agcggatgcc
3661 gggagcagac aagcccgtca gggcgcgtca gcgggtgttg gcgggtgtcg gggctggctt
3721 aactatgcgg catcagagca gattgtactg agagtgcacc atatgcggtg tgaataaccg
3781 cacagatgcg taaggagaaa ataccgcatc aggcgccatt cgccattcag gctgcgcaac
3841 gtgtgggaag ggcgatcggg gcgggcctct tcgctattac gccagctggc gaaaggggga
3901 gtgctgcaa ggcgattaag ttgggtaacg ccagggtttt cccagtcacg acgttgtaaa
3961 acgacggcca gtgaattaat tcgttgcagg acaggatgtg gtgcccgatg tgactagctc
4021 tttgctgcag gccgtcctat cctctggttc cgataagaga cccagaactc cggccccca
4081 ccgcccaccg ccacccccat acatatgtgg tacgcaagta agagtgcctg cgcatgcccc
4141 atgtgcccc ccaagagctt tgcattccat acaagtcccc aaagtggaga accgaaccaa
4201 ttcttcgagg gcagaacaaa agcttctgca cacgtctcca ctcgaatttg gagccggccg
4261 gcgtgtgcaa aagaggtgaa tcgaacgaaa gaccctgtg taaagcccg tttccaaaat
4321 gtataaaacc gagagcatct ggccaatgtg catcagttgt ggtcagcagc aaaatcaagt
4381 gaatcatctc agtgcaacta aaggggggat ctagatcggG GTACCTActa gcgctaccgg
4441 actcagatct cgagctcgcc accATGAGGC TTCGGGAGCC GTCCTGAGC GGCAGCGCCG
4501 CGATGCCAGG CGCGTCCCTA CAGCGGCCT GCCCCTGCT CGTGGCCGTC TGCCTCTGC
4561 ACCTTGCGT CACCCTCGTT TACTACCTGG CTGGCCGCGA CCTGAGCCGC CTGCCCAAC
4621 TGGTCGAGT CTCCACACCG CTGCAGTCGA CGGTACCGCG GCCCCGGAT CCACCGGTcg
4681 ccaccATGGT GAGCAAGGGC GAGGAGCTGT TCACCGGGGT GGTGCCATC CTGGTCGAGC
```

```
4741 TGGACGGCGA CGTAAACGGC CACAAGTTCA GCGTGTCCGG CGAGGGCGAG GGCGATGCCA
4801 CCTACGGCAA GCTGACCCTG AAGTTCATCT GCACCACCGG CAAGCTGCCC GTGCCCTGGC
4861 CCACCCTCGT GACCACCCTG ACCTACGGCG TGCAGTGCTT CAGCCGCTAC CCCGACCACA
4921 TGAAGCAGCA CGACTTCTTC AAGTCCGCCA TGCCCGAAGG CTACGTCCAG GAGCGCACCA
4981 TCTTCTTCAA GGACGACGGC AACTACAAGA CCCGCGCCGA GGTGAAGTTC GAGGGCGACA
5041 CCCTGGTGAA CCGCATCGAG CTGAAGGGCA TCGACTTCAA GGAGGACGGC AACATCCTGG
5101 GGCACAAGCT GGAGTACAAC TACAACAGCC ACAACGTCTA TATCATGGCC GACAAGCAGA
5161 AGAACGGCAT CAAGGTGAAC TTCAAGATCC GCCACAACAT CGAGGACGGC AGCGTGCAGC
5221 TCGCCGACCA CTACCAGCAG AACACCCCA TCGGCGACGG CCCCCTGCTG CTGCCCGACA
5281 ACCACTACCT GAGCACCCAG TCCGCCCTGA GCAAAGACCC CAACGAGAAG CGCGATCACA
5341 TGGTCCTGCT GGAGTTCGTG ACCGCCGCCG GGATCACTCT CGGCATGGAC GAGCTGTACA
5401 AGTAAagcgg ccgctcgagt ctagagggcc cttcgaaggt aagcctatcc ctaaccctct
5461 cctcgggtctc gattctacgc gtaccggtca tcatcaccat caccattgag tttaaaccg
5521 ctgatcagcc tcgactgtgc cttctaagat ccagacatga taagatacat tgatgagttt
5581 ggacaaacca caactagaat gcagtgaaaa aatgcttta tttgtgaaat ttgtgatgct
5641 attgctttat ttgtaacat t
```

//

pMT-ManII::EGFP

LOCUS pMT\_ManII\_EGFP 5805 bp ds-DNA circular 28-FEB-2020

DEFINITION pMT-puro Sequencing Result

ORGANISM other sequences; artificial sequences; vectors.

COMMENT pMT-puro from 1 to 4724

COMMENT ApEinfo:methylated:1

FEATURES Location/Qualifiers

gene 4833..5546

/gene="EGFP"

/translation="MVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLT

LKFICTTGKLPVPWPTLVTTLTYGVCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFK

DDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNNSHNVYIMADKQKN

GIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDH

MVLLFVTAAGITLGMDELYKSGLRSRAQASNSAVDGTAGPGSTGSR\*"

```

        /label=EGFP
        /ApEinfo_fwdcolor=#66ff66
        /ApEinfo_revcolor=#ffffcc
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
misc_feature    5558..5563
        /label=XhoI
        /ApEinfo_fwdcolor=#ffcc66
        /ApEinfo_revcolor=#ffcc66
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
misc_feature    complement(4875..4896)

/translation="MVSKGEELFTGVVPIVELDGDVNGHKFSVSGEGEGDATYGKLT
LKFICTTGKLPVPWPTLVTTLTYGVCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFK
DDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNNSHNVYIMADKQKN
GIKVNFKIRHNIEDGSVQLADHYQNTPIGDGPVLLPDNHYLSTQSALS KDPNEKRDH
        MVLLEFVTAAGITLGMDELYKSGLRSRAQASNSAVDGTAGPGSTGSR*"
        /label=EGFP_N_primer
        /ApEinfo_fwdcolor=#ffcc66
        /ApEinfo_revcolor=#ffcc66
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
misc_feature    5013..5042

/translation="MVSKGEELFTGVVPIVELDGDVNGHKFSVSGEGEGDATYGKLT
LKFICTTGKLPVPWPTLVTTLTYGVCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFK
DDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNNSHNVYIMADKQKN
GIKVNFKIRHNIEDGSVQLADHYQNTPIGDGPVLLPDNHYLSTQSALS KDPNEKRDH
        MVLLEFVTAAGITLGMDELYKSGLRSRAQASNSAVDGTAGPGSTGSR*"

```

```

        /label=Y66 (EGFP)
        /ApEinfo_fwdcolor=#ffcc66
        /ApEinfo_revcolor=#ffcc66
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
misc_feature    5483..5504

/translation="MVSKGEELFTGVVPIVELDGDVNGHKFSVSGEGEGDATYGKLT

LKFICTTGKLPVPWPTLVTTLTYGVCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFK

DDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNNSHNVYIMADKQKN

GIKVNFKIRHNIEDGSVQLADHYQNTPIGDGPVLLPDNHYLSTQSALS KDPNEKRDH
        MVLLEFVTAAGITLGMDELYKSGLRRAQASNSAVDGTAGPGSTGSR*"
        /label=EGFP_C_primer
        /ApEinfo_fwdcolor=#ffcc66
        /ApEinfo_revcolor=#ffcc66
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
misc_feature    4464..4811
        /label=New Feature
        /ApEinfo_fwdcolor=cyan
        /ApEinfo_revcolor=#00ff00
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
misc_feature    complement(3820..3975)
        /label=lacZ_a
        /ApEinfo_fwdcolor=#ff3600
        /ApEinfo_revcolor=#ff3600
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
promoter        3956..3972
        /label=M13_forward20_primer
        /ApEinfo_fwdcolor=#ccffed
        /ApEinfo_revcolor=#ccffed
```

```

    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    3941..3963
    /label=M13_pUC_fwd_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    complement(3606..3628)
    /label=pGEX_3_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
promoter        complement(3419..3447)
    /label=AmpR_promoter
    /ApEinfo_fwdcolor=#ccffed
    /ApEinfo_revcolor=#ccffed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
gene            complement(2517..3377)
    /gene="Ampicillin"
    /label=Ampicillin
    /ApEinfo_fwdcolor=#ffffcc
    /ApEinfo_revcolor=#ffffcc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
rep_origin      complement(1743..2362)

/translation="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGY
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLTTIGGPKELTAFLHNMGDHSVTRL
DRWEPELNEAIPNDERDTMPVAMATTLRKLTTGELLTLASRQQLIDWMEADKVAGPL
```



```
LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
      EIGASLIKHW*"
      /label=pBR322_origin
      /ApEinfo_fwdcolor=pink
      /ApEinfo_revcolor=pink
      /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
      width 5 offset 0
promoter      complement(1405..1434)

/translation="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGY
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHSVTRL
DRWEPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPL

LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
      EIGASLIKHW*"
      /label=lac_promoter
      /ApEinfo_fwdcolor=#ccffed
      /ApEinfo_revcolor=#ccffed
      /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
      width 5 offset 0
misc_feature  complement(1369..1391)

/translation="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGY
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHSVTRL
DRWEPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPL

LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
```

```
EIGASLIKHW*"
/label=M13_pUC_rev_primer
/ApEinfo_fwdcolor=#ff3600
/ApEinfo_revcolor=#ff3600
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
promoter      complement(1352..1370)

/translation="MSIQHFRVALIPFFAAFLPVFHAHPETLVKVKDAEDQLGARVGY
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLTTIGGPKELTAFLHNMGDHSVTRL
DRWEPELNEAIPNDERDTMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPL
LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
EIGASLIKHW*"
/label=M13_reverse_primer
/ApEinfo_fwdcolor=#ccffed
/ApEinfo_revcolor=#ccffed
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
gene          complement(143..742)
/label=puro (variant)
/translation="MGSCAPFGRALRVVGRASGTGLAGHAPGARSFGHLDVGGDGAE
PLVEGEVAGRGGLEGGHPGALGRLHSGEHDGAAQTLALVVGRDADGGQEPRGLLGPV
RRQEAFLHLLLRGQPETAQLGHARADLGEHRPRFDALRRGPDRHRGAVVRDPHLADVEP
DAREEEFLQLGDPLDVAVRVDGVARGGVGERGEGAYGPGDVVAGGEAHRGLVLGHG
RSSPCEGSGAWVRGWWHRHSWRPTCRHASYRIPAARGICCNL*"
/label=puro (variant)
/ApEinfo_fwdcolor=#ffffcc
```

```

    /ApEinfo_revcolor=#ffffcc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature    3982..4349
    /label=MT-promoter
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    3955..3972
    /label=M13-fwd
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=green
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    complement(1352..1372)
    /label=M13-rev
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=green
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature    complement(1574..1577)
    /label=Sac1 GAGCT/C (1/2)
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
rep_origin      complement(1740..2422)
    /label=ColE1 origin
    /ApEinfo_fwdcolor=gray50
    /ApEinfo_revcolor=gray50
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_recomb     1574..1580
    /label=BstQI GCTCTTCN/NNN
    /ApEinfo_fwdcolor=#ffb500
```

```

/ApEinfo_revcolor=#ff7e07
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
CDS      complement(3816..3884)
/label=LacZ alpha
/ApEinfo_fwdcolor=#6495ed
/ApEinfo_revcolor=#6495ed
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_binding  complement(1378..1400)
/label=Lac0
/ApEinfo_fwdcolor=#6495ed
/ApEinfo_revcolor=#6495ed
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
CDS      complement(2520..3179)
/label=AmpR
/ApEinfo_fwdcolor=yellow
/ApEinfo_revcolor=yellow
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind  3929..3952
/label=M13F
/ApEinfo_fwdcolor=#0a00ff
/ApEinfo_revcolor=#0a00ff
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind  3955..3972
/label=M13F_GW
/ApEinfo_fwdcolor=#ff00bd
/ApEinfo_revcolor=#ff0003
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind  complement(1352..1372)
/label=M13R_GW
/ApEinfo_fwdcolor=#ff00bd
```

```
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind    3491..3508
    /label=pQE60-F
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature    complement(843..1201)
    /label=Copia Promoter?
    /ApEinfo_fwdcolor=#cde7f7
    /ApEinfo_revcolor=#cde7f7
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature    4385..4402
    /label=Metallothionein_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature    5582..5623
    /label=V5
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature    complement(5790..5797)
    /label=New Feature(1)
    /ApEinfo_label=New Feature
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature    complement(5716..5735)
    /label=EBV_rev_primer
```

```
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    complement(5673..5690)
    /label=BGH_rev_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    5633..5650
    /label=6xHis
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    complement(5761..5767)
    /label=New Feature(2)
    /ApEinfo_label=New Feature
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
```

ORIGIN

```
1 ataagctgca ataaacaagt tctagagtcg gtgggcctcg ggggcgggtg cggggtcggc
61 ggggccgccc cgggtggctt cggtcggagc catggggtcg tgcgctcctt tcggtcgggc
121 gctgcgggtc gtggggcggg cgtcaggcac cgggcttgcg ggtcatgcac caggtgcgcg
181 gtccttcggg cacctcgacg tcggcgggtg cggtgaagcc gagccgctcg tagaagggga
241 ggttgccggg cgcgagggtc tccaggaagg cgggcacccc ggcgcgctcg gccgcctcca
301 ctccggggag cagcagggcg ctgcccagac cttgcccctg gtggtcgggc gagacgccga
361 cggtggccag gaaccacgcg ggctccttgg gccggtgcg cgccaggagg ctttccatct
421 gttgctgcgc ggccagccgg gaaccgctca actcggccat gcgcgggccg atctcggcga
481 acaccgcccc cgcttcgacg ctctccggcg tggtcagac cgccaccgcg gcgcccgtct
541 ccgacacca caccttgccg atgtcgagcc cgacgcgctg gaggaagagt tcttgcagct
601 cggtgaccgc ctcgatgtgg cggtcgggtg cgacgggtgt gcgctggcg gggtagtcgg
661 cgaacgcggc ggcgagggtg cgtacggccc gggggacgct gtcgcggtg gcgaggcgca
```

721 ccgtgggctt gtactcggtc atggaaggtc gtctccttgt gaggggtcag gggcgtgggt  
781 caggggatgg tggcggcacc ggtcgtggcg gccgacctgc aggcattgcaa gctatcgaat  
841 tcctgcagcc cgggggatct gttgtaattt ataatttata tttcctttct taataataa  
901 ataaatagtc aagtttatgt ttgagtttta tgatttatat ttttaagttat ttcaactgca  
961 acaccagcac cacgacctac ttacagcaaa aaacgtacaa gaaggaaaga aggaataaaa  
1021 agagtgggat tctcttacia tatgttttat ggcataaaag gtgtggccat tcatatcaaa  
1081 tataaagtag tgttgtttaa cgttactttt gtaggttgaa tagtatattc caacagatga  
1141 tgaggggttc ccaatcctaa acccatttgc cgttcccaga agcatgaaac caccacgcac  
1201 cggatcctct agaacaacia caattgcatt ctttttatgt ttcaggttca gggggagggtg  
1261 tgggagggtt tttaaagcaa gtaaaacctc taaaaatgtg gtatggctga ttatgatcag  
1321 tcgacctgca ggcattgcaag cttggcgtaa tcatggcat agctgtttcc tgtgtgaaat  
1381 tgttatccgc tcacaattcc acacaacata cgagccggaa gcataaagtg taaagcctgg  
1441 ggtgcctaag gaggagcta actcacatta attgcgttgc gctcactgcc cgctttccag  
1501 tcgggaaacc tgcctgcca gctgcattaa tgaatcggcc aacgcgcggg gagaggcggg  
1561 ttgcgtattg ggcgtcttc cgcttctcgc ctactgact cgctgcgctc ggtcgttcgg  
1621 ctgcggcgag cggtatcagc tcaactcaag gcggtataac ggttatccac agaatcaggg  
1681 gataacgcag gaaagaacat gtgagcaaaa ggccagcaaa aggccaggaa ccgtaaaaag  
1741 gccgcgttgc tggcgttttt ccataggctc cgccccctg acgagcatca caaaaatcga  
1801 cgctcaagtc agagggtggcg aaacccgaca ggactataaa gataccaggc gtttcccct  
1861 ggaagctccc tcgtgcgctc tctgttccg accctgccgc ttaccggata cctgtccgcc  
1921 tttctccctt cgggaagcgt ggcgtttct catagctcac gctgtaggta tctcagttcg  
1981 gtgtaggtcg ttcgctcaa gctgggctgt gtgcacgaac cccccgtca gcccgaccgc  
2041 tgcgccttat ccgtaacta tcgtctttag tccaacccgg taagacacga cttatcgcca  
2101 ctggcagcag ccaactgtaa caggattagc agagcgaggc atgtaggcgg tgctacagag  
2161 ttcttgaagt ggtggcctaa ctacggctac actagaagaa cagtatttgg tatctgcgct  
2221 ctgctgaagc cagttacctt cggaaaaaga gttggtagct cttgatccgg caaacaacc  
2281 accgctggta gcggtggtt ttttgttgc aagcagcaga ttacgcgcag aaaaaagga  
2341 tctcaagaag atcctttgat cttttctacg gggctgacg ctacgtggaa cgaaaactca  
2401 cgtaagga ttttggctat gagattatca aaaaggatct tcacctagat ctttttaaat  
2461 taaaaatgaa gttttaaatc aatctaaagt atatatgagt aaacttggtc tgacagttac  
2521 caatgcttaa tcagtgaggc acctatctca gcgatctgtc ttttctgtc atccatagtt  
2581 gcctgactcc ccgtcgtgta gataactacg atacgggagg gcttaccatc tggccccagt  
2641 gctgcaatga taccgcgaga cccacgctca ccggctccag atttatcagc aataaaccag  
2701 ccagccggaa gggccgagcg cagaagtggc cctgcaactt tatccgcctc catccagtct  
2761 attaatgtt gccgggaagc tagagtaagt agttcgccag ttaatagttt gcgcaacggt  
2821 gttgccattg ctacaggcat cgtgggtgca cgctcgtcgt ttggtatggc ttcattcagc

2881 tccggttccc aacgatcaag gcgagttaca tgatcccca tgttgtgcaa aaaagcggtt  
2941 agctccttcg gtcctccgat cgttgtcaga agtaagttgg ccgcagtgtt atcactcatg  
3001 gttatggcag cactgcataa ttctcttact gtcatgccat ccgtaagatg cttttctgtg  
3061 actggtgagt actcaaccaa gtcattctga gaatagtgta tgcggcgacc gagttgctct  
3121 tgcccggcgt caatacggga taataccgcg ccacatagca gaactttaaa agtgctcatc  
3181 attgaaaaac gttcttcggg gcgaaaactc tcaaggatct taccgctgtt gagatccagt  
3241 tcgatgtaac ccactcgtgc acccaactga tcttcagcat cttttacttt caccagcgtt  
3301 tctgggtgag caaaaacagg aaggcaaaat gccgcaaaa agggataaag ggcgacacgg  
3361 aatggtgaa tactcatact cttccttttt caatattatt gaagcattta tcagggttat  
3421 tgtctcatga gcgatacat atttgaatgt atttagaaaa ataaacaaat aggggttccg  
3481 cgcacatttc cccgaaaagt gccacctgac gtctaagaaa ccattattat catgacatta  
3541 acctataaaa ataggcgtat cacgaggccc tttcgtctcg cgcgtttcgg tgatgacggt  
3601 gaaaacctct gacacatgca gctcccggag acggtcacag cttgtctgta agcggatgcc  
3661 gggagcagac aagcccgtca gggcgcgtca gcgggtgttg gcgggtgtcg gggctggcct  
3721 aactatgcgg catcagagca gattgtactg agagtgcacc atatgcggtg tgaataaccg  
3781 cacagatgcg taaggagaaa ataccgcatc aggcgccatt cgccattcag gctgcgcaac  
3841 tgttgggaag ggcgatcggg gcgggcctct tcgctattac gccagctggc gaaaggggga  
3901 tgtgctgcaa ggcgattaag ttgggtaacg ccagggtttt cccagtcacg acgttgtaaa  
3961 acgacggcca gtgaattaat tcgttgcagg acaggatgtg gtgcccgatg tgactagctc  
4021 tttgctgcag gccgtcctat cctctggttc cgataagaga cccagaactc cggcccccca  
4081 ccgcccaccg ccacccccat acatatgtgg tacgcaagta agagtgcctg cgcatgcccc  
4141 atgtgcccc ccaagagctt tgcattccat acaagtcccc aaagtggaga accgaaccaa  
4201 ttcttcgagg gcagaacaaa agcttctgca cacgtctcca ctcgaatttg gagccggccg  
4261 gcgtgtgcaa aagaggtgaa tcgaacgaaa gaccctgtgt taaagccgcg tttccaaaat  
4321 gtataaaacc gagagcatct ggccaatgtg catcagttgt ggtcagcagc aaaatcaagt  
4381 gaatcatctc agtgcaacta aaggggggat ctagatcggg gtacctacta gcgctaccgg  
4441 actcagatct cgagctcgc accATGAAGT TAAGTCGCCA GTTCACCGTG TTTGGCAGCG  
4501 CGATCTTCTG CGTCGTAATC TTCTACTCT ACCTGATGCT GGACAGGGGT CACTTGGACT  
4561 ACCCTCGGGG CCCGCGCCAG GAGGGCTCCT TTCCGAGGG CCAGCTTCA ATATTGCAAG  
4621 AAAAGATTGA CCATTTGGAG CGTTTGCTCG CTGAGAACAA CGAGATTATC TCAAATATCA  
4681 GAGACTCAGT CATCAACCTG AGCGAGTCTG TGGAGGACGG CCCGCGGGG TCACCAGGCA  
4741 ACGCCAGCCA AGGCTCCATC CACCTCCACT CGCCACAGTT GGCCCTGCAG GCTGACCCCA  
4801 GAGACTGTTT GGATCCACCG GTcgccaccA TGGTGAGCAA GGGCGAGGAG CTGTTACCG  
4861 GGGTGGTGCC CATCCTGGTC GAGCTGGACG GCGACGTAAG CGGCCACAAG TTCAGCGTGT  
4921 CCGGCGAGGG CGAGGGCGAT GCCACCTACG GCAAGCTGAC CCTGAAGTTC ATCTGCACCA  
4981 CCGGCAAGCT GCCCGTGCC TGGCCACCC TCGTGACCAC CCTGACCTAC GCGTGCAGT



```

5041 GCTTCAGCCG CTACCCCGAC CACATGAAGC AGCACGACTT CTTCAAGTCC GCCATGCCCG
5101 AAGGCTACGT CCAGGAGCGC ACCATCTTCT TCAAGGACGA CGGCAACTAC AAGACCCGCG
5161 CCGAGGTGAA GTTCGAGGGC GACACCCTGG TGAACCGCAT CGAGCTGAAG GGCATCGACT
5221 TCAAGGAGGA CGGCAACATC CTGGGGCACA AGCTGGAGTA CAACTACAAC AGCCACAACG
5281 TCTATATCAT GGCCGACAAG CAGAAGAACG GCATCAAGGT GAACTTCAAG ATCCGCCACA
5341 ACATCGAGGA CGGCAGCGTG CAGCTCGCCC ACCACTACCA GCAGAACACC CCCATCGGCG
5401 ACGGCCCCGT GCTGCTGCCC GACAACCACT ACCTGAGCAC CCAGTCCGCC CTGAGCAAAG
5461 ACCCCAACGA GAAGCGCGAT CACATGGTCC TGCTGGAGTT CGTGACCGCC GCCGGGATCA
5521 CTCTCGGCAT GGACGAGCTG TACAAGTAAa GCGGCCGCTC GAGTctagaG GGCCctcgA
5581 aggtaagcct atccctaacc ctctcctcgg tctcgattct acgcgtaccg gtcacatca
5641 ccatcaccat tgagtttaaa cccgctgac agcctcgact gtgccttcta agatccagac
5701 atgataagat acattgatga gtttgacaa accacaacta gaatgcagtg aaaaaaatgc
5761 tttatttgatg aaatttgatg tgctattgct ttatttgtaa ccatt

```

//

**pMT-ST::EGFP**

LOCUS pMT\_ST\_EGFP 5610 bp ds-DNA circular 28-FEB-2020

DEFINITION pMT-puro Sequencing Result

ORGANISM other sequences; artificial sequences; vectors.

COMMENT pMT-puro from 1 to 4724

COMMENT ApEinfo:methylated:1

FEATURES Location/Qualifiers

misc\_feature complement(5521..5540)  
 /label=EBV\_rev\_primer  
 /ApEinfo\_fwdcolor=#ff3600  
 /ApEinfo\_revcolor=#ff3600  
 /ApEinfo\_graphicformat=arrow\_data {{0 1 2 0 0 -1} {} 0}  
 width 5 offset 0

misc\_feature complement(5478..5495)  
 /label=BGH\_rev\_primer  
 /ApEinfo\_fwdcolor=#ff3600  
 /ApEinfo\_revcolor=#ff3600  
 /ApEinfo\_graphicformat=arrow\_data {{0 1 2 0 0 -1} {} 0}  
 width 5 offset 0

misc\_feature 5438..5455  
 /label=6xHis

```

    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    complement(3820..3975)
    /label=lacZ_a
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    5387..5428
    /label=V5
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
promoter        3956..3972
    /label=M13_forward20_primer
    /ApEinfo_fwdcolor=#ccffed
    /ApEinfo_revcolor=#ccffed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    3941..3963
    /label=M13_pUC_fwd_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature    complement(3606..3628)
    /label=pGEX_3_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
promoter        complement(3419..3447)
    /label=AmpR_promoter
```

```

    /ApEinfo_fwdcolor=#ccffed
    /ApEinfo_revcolor=#ccffed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
gene      complement(2517..3377)
    /gene="Ampicillin"
    /label=Ampicillin
    /ApEinfo_fwdcolor=#ffffcc
    /ApEinfo_revcolor=#ffffcc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
rep_origin  complement(1743..2362)

/translation="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGY
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHVTRL
DRWEPELNEAIPNDERDTMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPL
LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
    EIGASLIKHW*"
    /label=pBR322_origin
    /ApEinfo_fwdcolor=pink
    /ApEinfo_revcolor=pink
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
promoter   complement(1405..1434)

/translation="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGY
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHVTRL
```

```
DRWEPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPL

LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
    EIGASLIKHW*"
    /label=lac_promoter
    /ApEinfo_fwdcolor=#ccffed
    /ApEinfo_revcolor=#ccffed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    4464..4595
    /label=ST
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    complement(1369..1391)

/translation="MSIQHFRVALIPFFAAFLPVAHPETLVKVKDAEDQLGARVGY

IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE

YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLTTIGGPKELTAFLHNMGDHVTRL

DRWEPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPL

LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
    EIGASLIKHW*"
    /label=M13_pUC_rev_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
promoter        complement(1352..1370)

/translation="MSIQHFRVALIPFFAAFLPVAHPETLVKVKDAEDQLGARVGY
```

```
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLTTIGGPKELTAFLHNMGDHSVTRL
DRWEPELNEAIPNDERDTTMPVAMATTLRKLTLGELLTLASRQQLIDWMEADKVAGPL
LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
    EIGASLIKHW*"
    /label=M13_reverse_primer
    /ApEinfo_fwdcolor=#ccffed
    /ApEinfo_revcolor=#ccffed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
gene      complement(143..742)
    /gene="puro (variant)"

/translation="MGSCAPFGRALRVVGRASGTGLAGHAPGARSFGHLDVGGDGEAE
PLVEGEVAGRGGLEGGHPGALGRLHSGEHDGAAQTLALVVRDADGGQEPRGLLGPV
RRQEA FHLLL RGQP GTAQLGHARADLGEHRPRFDALRRGPD RHRGAVVRDPHLADVEP
DAREEEFLQLGDPLDVAVRVDGVARGGVVGERGGEGAYGPGDVVAGGEAHRGLVLGHG
    RSPCEGSGAWVRGWRHRSWRPTCRHASYRIPAARGICCNL*"
    /label=puro (variant)
    /ApEinfo_fwdcolor=#ffffcc
    /ApEinfo_revcolor=#ffffcc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature 3982..4349
    /label=MT-promoter
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature 5363..5368
```

```

        /label=XhoI
        /ApEinfo_fwdcolor=#ffcc66
        /ApEinfo_revcolor=#ffcc66
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
gene      4638..5351
        /gene="EGFP"

/translation="MVSKGEELFTGVVPIVELDGDVNGHKFSVSGEGEGDATYGKLT
LKFICTTGKLPVPWPTLVTTLTYGVCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFK
DDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNNSHNVYIMADKQKN
GIKVNFKIRHNIEDGSVQLADHYQNTPIGDGPVLLPDNHYLSTQSALS KDPNEKRDH
        MVLLEFVTAAGITLGMDELYKSGLRSAQASNSAVDGTAGPGSTGSR*"
        /label=EGFP
        /ApEinfo_fwdcolor=#66ff66
        /ApEinfo_revcolor=#ffffcc
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
primer_bind 3955..3972
        /label=M13-fwd
        /ApEinfo_fwdcolor=cyan
        /ApEinfo_revcolor=green
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
        width 5 offset 0
misc_feature complement(4680..4701)

/translation="MVSKGEELFTGVVPIVELDGDVNGHKFSVSGEGEGDATYGKLT
LKFICTTGKLPVPWPTLVTTLTYGVCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFK
DDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNNSHNVYIMADKQKN
GIKVNFKIRHNIEDGSVQLADHYQNTPIGDGPVLLPDNHYLSTQSALS KDPNEKRDH
```

```
MVLLFEVTAAGITLGMDELYKSGRLRSRAQASNSAVDGTAGPGSTGSR*"
/label=EGFP_N_primer
/ApEinfo_fwdcolor=#ffcc66
/ApEinfo_revcolor=#ffcc66
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    complement(1352..1372)
/label=M13-rev
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=green
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature    complement(1574..1577)
/label=Sac1 GAGCT/C (1/2)
/ApEinfo_fwdcolor=cyan
/ApEinfo_revcolor=#00ff00
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature    5288..5309

/translation="MVSKGEELFTGVVPIVELDGDVNGHKFSVSGEGEGDATYGKLT
LKFICTTGKLPVPWPTLVTTLTYGVCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFK
DDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNNSHNVYIMADKQKN
GIKVNFKIRHNIEDGSVQLADHYQNTPIGDGPVLLPDNHYLSTQSALS KDPNEKRDH
MVLLFEVTAAGITLGMDELYKSGRLRSRAQASNSAVDGTAGPGSTGSR*"
/label=EGFP_C_primer
/ApEinfo_fwdcolor=#ffcc66
/ApEinfo_revcolor=#ffcc66
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
rep_origin    complement(1740..2422)
/label=ColE1 origin
/ApEinfo_fwdcolor=gray50
```

```

/ApEinfo_revcolor=gray50
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_recomb    1574..1580
/ApEinfo_revcolor=gray50
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_recomb    1574..1580
/label=BstQI GCTCTTCN/NNN
/ApEinfo_fwdcolor=#ffb500
/ApEinfo_revcolor=#ff7e07
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
CDS            complement(3816..3884)
/label=LacZ alpha
/ApEinfo_fwdcolor=#6495ed
/ApEinfo_revcolor=#6495ed
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_binding   complement(1378..1400)
/label=Lac0
/ApEinfo_fwdcolor=#6495ed
/ApEinfo_revcolor=#6495ed
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
CDS            complement(2520..3179)
/label=AmpR
/ApEinfo_fwdcolor=yellow
/ApEinfo_revcolor=yellow
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind    3929..3952
/label=M13F
/ApEinfo_fwdcolor=#0a00ff
/ApEinfo_revcolor=#0a00ff
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind    3955..3972
/label=M13F_GW
/ApEinfo_fwdcolor=#ff00bd
```



```

    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    complement(1352..1372)
    /label=M13R_GW
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    3491..3508
    /label=pQE60-F
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature    complement(843..1201)
    /label=Copia Promoter?
    /ApEinfo_fwdcolor=#cde7f7
    /ApEinfo_revcolor=#cde7f7
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature    4385..4402
    /label=Metallothionein_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature    complement(5595..5602)
    /label=New Feature
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature    complement(5566..5572)
    /label=New Feature(1)
    /ApEinfo_label=New Feature
```

```
/ApEinfo_fwdcolor=cyan  
/ApEinfo_revcolor=#00ff00  
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1} {} 0}  
width 5 offset 0
```

ORIGIN

```
1 ataagctgca ataaacaagt tctagagtcg gtgggcctcg ggggcgggtg cggggtcggc  
61 ggggccgccc cgggtggctt cggtcggagC CATGGggtcg tgcgctcctt tcggtcgggc  
121 gctgagggtc gtggggcggg cgTCAGGCAC CGGGCTTGCG GGTCATGCAC CAGGTGCGCG  
181 GTCCTTCGGG CACCTCGACG TCGGCGGTGA CGGTGAAGCC GAGCCGCTCG TAGAAGGGGA  
241 GGTTGCGGGG CGCGGAGGTC TCCAGGAAGG CGGGCACCCC GGCGCGCTCG GCCGCCTCCA  
301 CTCCGGGGAG CACGACGGCG CTGCCAGAC CCTTGCCCTG GTGGTCGGGC GAGACGCCGA  
361 CGGTGGCCAG GAACCACGCG GGCTCCTTGG GCCGGTGGCG CGCCAGGAGG CCTTCCATCT  
421 GTTGTGCGC GGCCAGCCGG GAACCGCTCA ACTCGCCAT GCGCGGGCCG ATCTCGGCGA  
481 ACACCGCCCC CGCTTCGACG CTCTCCGGCG TGGTCCAGAC CGCCACCGCG GCGCCGTCGT  
541 CCGCGACCCA CACCTTGCCG ATGTCGAGCC CGACGCGCGT GAGGAAGAGT TCTTGCAGCT  
601 CGGTGACCCG CTCGATGTGG CGGTCCGGGT CGACGGTGTG GCGCGTGGCG GGGTAGTCGG  
661 CGAACGCGGC GCGGAGGGTG CGTACGGCCC GGGGGACGTC GTCGCGGGTG GCGAGGCGCA  
721 CCGTGGGCTT GACTCGGTC ATggaagtc gtctccttgt gaggggtcag gggcgagggt  
781 caggggatgg tggcggcacc ggtcgtggcg gccgacctgc aggcattgca gctatcGAAT  
841 TCCTGCAGCC CGGGGGATCT GTTGAATTT ATAATTTATA TTTCTTTCT TAATAAATAA  
901 ATAAATAGTC AAGTTTATGT TTGAGTTTTA TGATTTATAT TTTAAGTTAT TTCAACTGCA  
961 ACACCAGCAC CACGACCTAC TTACAGCAA AAACGTACAA GAAGGAAAGA AGGAATAAAA  
1021 AGAGTGGTAT TCTCTTACAA TATGTTTTAT GGCATAAAAG GTGTGGCCAT TCATATCAAA  
1081 TATAAAGTAG TGTTGTTTAA CGTTACTTTT GTAGGTTGAA TAGTATATTC CAACAGATGA  
1141 TGAGGGGTTT CCAATCCTAA ACCCATTTCG CGTTCCAGAG AGCATGAAAC CACCACGCAC  
1201 cGGATCCTCT AGAacaacaa caattgcatt cattttatgt ttcagggtca gggggagggtg  
1261 tgggaggttt tttaaagcaa gtaaacctc tacaatgtg gtatggctga ttatgatcag  
1321 tcgacctgca ggcattgAAG CTTggcgtaa tcatggcat agctgtttcc tgtgtgaaat  
1381 tgttatccgc tcacaattcc acacaacata cgagccgga gcataaagt taaagcctgg  
1441 ggtgccta at gagtgagcta actcacatta attgcgttgc gctcactgcc cgctttccag  
1501 tcgggaaacc tgcgtgcca gctgcattaa tgaatcggc aacgcgcggg gagaggcggg  
1561 ttgcgtattg ggcGCTCTTC cgcttcctcg ctactgact cgctgcgctc ggtcgttcgg  
1621 ctgcggcgag cggtatcagc tactcaaag gcggtaatac ggttatccac agaatcaggg  
1681 gataacgcag gaaagaacat gtgagcaaaa ggccagcaaa aggccaggaa ccgtaaaaag  
1741 gccgcgttgc tggcgttttt ccataggctc cgccccctg acgagcatca caaaaatcga  
1801 cgctcaagtc agagggtggcg aaacccgaca ggactataaa gataaccaggc gtttccccct
```

1861 ggaagctccc tcgtgcgctc tcctgttccg accctgccgc ttaccggata cctgtccgcc  
1921 tttctccctt cgggaagcgt ggcgctttct catagctcac gctgtaggta tctcagttcg  
1981 gtgtaggtcg ttcgctccaa gctgggctgt gtgcacgaac cccccgttca gcccgaccgc  
2041 tgcgccttat ccggtaaacta tcgtcttgag tccaaccgga taagacacga cttatcgcca  
2101 ctggcagcag ccaactggtaa caggattagc agagcgaggt atgtaggcgg tgctacagag  
2161 ttcttgaagt ggtggcctaa ctacggctac actagaagaa cagtatttgg tatctgcgct  
2221 ctgctgaagc cagttacctt cggaaaaaga gttggtagct cttgatccgg caaacaacc  
2281 accgctggta gcggtggttt ttttgtttgc aagcagcaga ttacgcgcag aaaaaagga  
2341 tctcaagaag atcctttgat cttttctacg gggcttgacg ctacagtgaa cgaaaactca  
2401 cgtaagga ttttggctcat gagattatca aaaaggatct tcacctagat ctttttaaat  
2461 taaaaatgaa gttttaaatc aatctaaagt atatatgagt aaacttggtc tgacagttac  
2521 caatgcttaa tcagtgaggc acctatctca gcgatctgtc tatttcgttc atccatagtt  
2581 gcctgactcc ccgtcgtgta gataactacg atacgggagg gcttaccatc tggccccagt  
2641 gctgcaatga taccgcgaga cccacgctca ccggctccag atttatcagc aataaaccag  
2701 ccagccggaa gggccgagcg cagaagtggc cctgcaactt tatccgcctc catccagtct  
2761 attaatgtt gccgggaagc tagagtaagt agttcgccag ttaatagttt gcgcaacgtt  
2821 gttgccattg ctacaggcat cgtgggtgca cgctcgtcgt ttggtatggc ttcattcagc  
2881 tccggttccc aacgatcaag gcgagttaca tgatcccca tgttgtgcaa aaaagcggtt  
2941 agctccttcg gtcctccgat cgttgtcaga agtaagtggc ccgcagtgtt atcactcatg  
3001 gttatggcag cactgcataa ttctcttact gtcatgcat ccgtaagatg cttttctgtg  
3061 actggtgagt actcaaccaa gtcattctga gaatagtgta tgcggcgacc gagttgctct  
3121 tgcccggcgt caatacggga taataccgcg ccacatagca gaactttaa agtgctcatc  
3181 attggaaac gttcttcggg gcgaaaactc tcaaggatct taccgctgtt gagatccagt  
3241 tcgatgtaac ccaactcgtc acccaactga tcttcagcat cttttacttt caccagcgtt  
3301 tctgggtgag caaaaacagg aaggcaaat gccgcaaaa agggaatAAG GCGACACGG  
3361 AAATGttgaa tactcatact cttccttttt caatattatt gaagcattta tcagggttat  
3421 tgtctcatga gcggatacat atttgaatgt atttagaaaa ataaacaaat aggggttccg  
3481 gcacatttc cccgaaaagt gccacctgac gtctaagaaa ccattattat catgacatta  
3541 acctataaaa ataggcgtat cacgaggccc tttcgtctcg cgcgtttcgg tgatgacggt  
3601 gaaaacctct gacacatgca gctcccggag acggtcacag cttgtctgta agcggatgcc  
3661 gggagcagac aagcccgtca gggcgcgtca gcgggtgttg gcgggtgtcg gggctggctt  
3721 aactatgcgg catcagagca gattgtactg agagtgcacc atatgcggtg tgaataaccg  
3781 cacagatgcg taaggagaaa ataccgcatc aggcgccatt cgccattcag gctgcgcaac  
3841 tgttgggaag ggcgatcggg gcgggcctct tcgctattac gccagctggc gaaaggggga  
3901 tgtgctgcaa ggcgattaag ttgggtaacg ccagggtttt cccagtcacg acgttgtaaa  
3961 acgacggcca gtgaattaat tCGTTGCAGG ACAGGATGTG GTGCCCGATG TGACTAGCTC

```

4021 TTTGCTGCAG GCCGTCCTAT CCTCTGGTTC CGATAAGAGA CCCAGAACTC CGGCCCCCA
4081 CCGCCCACCG CCACCCCAT ACATATGTGG TACGCAAGTA AGAGTGCCTG CGCATGCCCC
4141 ATGTGCCCCA CCAAGAGCTT TGCATCCCAT ACAAGTCCCC AAAGTGGAGA ACCGAACCAA
4201 TTCTTCGCGG GCAGAACAAA AGCTTCTGCA CACGTCTCCA CTCGAATTTG GAGCCGCGCG
4261 GCGTGTGCAA AAGAGGTGAA TCGAACGAAA GACCCGTGTG TAAAGCCGCG TTTCCAAAAT
4321 GTATAAAACC GAGAGCATCT GGCCAATGTg catcagttgt ggtcagcagc aaaatcaagt
4381 gaatCATCTC AGTGCAACTA AAGGGGGGAT CTagatcggG GTACcACTA GcgctaccGG
4441 ACTCAGATCT CGAGctcgcc accATGATTC ACACCAACCT GAAGAAAAAG TTCAGTGCT
4501 GCGTCCTGGT CTTTCTTCTG TTTGCAGTCA TCTGTGTGTG GAAGGAAAAG AAGAAAGGGA
4561 GTTACTATGA TTCCTTTAAA TTGCAAACCA AGGGGTGCGAC GGTACCGCGG GCCCGGGATC
4621 CACCGGTcgc caccATGGTG AGCAAGGGCG AGGAGCTGTT CACCGGGGTG GTGCCATCC
4681 TGGTCGAGCT GGACGGCGAC GTAAACGGCC ACAAGTTCAG CGTGTCCGGC GAGGGCGAGG
4741 GCGATGCCAC CTACGGCAAG CTGACCCTGA AGTTCATCTG CACCACCGGC AAGTGCCCG
4801 TGCCCTGGCC CACCCTCGTG ACCACCCTGA CCTACGGCGT GCAGTGCTTC AGCCGCTACC
4861 CCGACCACAT GAAGCAGCAC GACTTCTTCA AGTCCGCCAT GCCCGAAGGC TACGTCCAGG
4921 AGCGCACCAT CTTCTTCAAG GACGACGGCA ACTACAAGAC CCGCGCCGAG GTGAAGTTCG
4981 AGGGCGACAC CCTGGTGAAC CGCATCGAGC TGAAGGGCAT CGACTTCAAG GAGGACGGCA
5041 ACATCCTGGG GCACAAGCTG GAGTACAAC ACAACAGCCA CAACGTCTAT ATCATGGCCG
5101 ACAAGCAGAA GAACGGCATC AAGGTGAACT TCAAGATCCG CCACAACATC GAGGACGGCA
5161 GCGTGCAGCT CGCCGACCAC TACCAGCAGA ACACCCCAT CGGCGACGGC CCCGTGCTGC
5221 TGCCCGACAA CCACTACCTG AGCACCCAGT CCGCCCTGAG CAAAGACCCC AACGAGAAGC
5281 GCGATCACAT GGTCTGCTG GAGTTCGTGA CCGCCGCCG GATCACTCTC GGCATGGACG
5341 AGCTGTACAA GTAAAGCGGC CGCTCGAGtc tagaGGGCC ttcgaaGGTA AGCCTATCCC
5401 TAACCCTCTC CTCGGTCTCG ATTCTACGcg taccggtCAT CATCACCATC ACCATTGAgt
5461 ttaaaccgc tgatcagcct cgactgtgcc ttctaagatc cagacatgat aagatacatt
5521 gatgagtttg gacaaaccac aactagaatg cagtgaaaa aatgctttat ttgtgaaatt
5581 tgtgatgcta ttgctttatt tgtaaccatt

```

//

pMT-GalT::EGFP-T2A-tdTomato::Rab11

LOCUS	pMT_GalT_EGFP_T2	7792 bp	ds-DNA	circular	28-FEB-2020
DEFINITION	pMT-puro Sequencing Result				
ORGANISM	other sequences; artificial sequences; vectors.				
COMMENT	pMT-GalT-GFP-T2A-tdTom-Rab6 from 1 to 7705				
COMMENT	pMT-GalT-EGFP-T2A-tdTom-Vamp3 from 1 to 7513				
COMMENT	pMT-EGFP-hVamp3 from 1 to 5841				

```
COMMENT    pMT-puro from 1 to 4724
COMMENT    ApEinfo:methylated:1
FEATURES   Location/Qualifiers
  misc_feature   complement(7703..7722)
                  /label=EBV_rev_primer
                  /ApEinfo_fwdcolor=#ff3600
                  /ApEinfo_revcolor=#ff3600
                  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                  width 5 offset 0
  misc_feature   6938..6961
                  /label=attB1
                  /ApEinfo_fwdcolor=cyan
                  /ApEinfo_revcolor=#00ff00
                  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                  width 5 offset 0
  misc_feature   4429..4605
                  /label=GaIT
                  /ApEinfo_fwdcolor=cyan
                  /ApEinfo_revcolor=#00ff00
                  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                  width 5 offset 0
  misc_feature   6942..6955
                  /label=att1_shared
                  /ApEinfo_fwdcolor=#ffc600
                  /ApEinfo_revcolor=#ffc600
                  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                  width 5 offset 0
  misc_feature   complement(7660..7677)
                  /label=BGH_rev_primer
                  /ApEinfo_fwdcolor=#ff3600
                  /ApEinfo_revcolor=#ff3600
                  /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                  width 5 offset 0
  gene          4654..5367
                  /gene="EGFP"
```

```
/translation="MVSKGEELFTGVVPIVELDGDVNGHKFSVSGEGEGDATYGKLT
LKFICTTGKLPVPWPTLVTTLTYGVCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFK
DDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNNSHNVYIMADKQKN
GIKVNFKIRHNIEDGSVQLADHYQNTPIGDGPVLLPDNHYLSTQSALS KDPNEKRDH
      MVLLEFVTAAGITLGMDELYKSGLRSAQASNSAVDGTAGPGSTGSR*"
      /label=EGFP
      /ApEinfo_fwdcolor=#66ff66
      /ApEinfo_revcolor=#ffffcc
      /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
      width 5 offset 0
misc_feature    complement(4696..4717)

/translation="MVSKGEELFTGVVPIVELDGDVNGHKFSVSGEGEGDATYGKLT
LKFICTTGKLPVPWPTLVTTLTYGVCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFK
DDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNNSHNVYIMADKQKN
GIKVNFKIRHNIEDGSVQLADHYQNTPIGDGPVLLPDNHYLSTQSALS KDPNEKRDH
      MVLLEFVTAAGITLGMDELYKSGLRSAQASNSAVDGTAGPGSTGSR*"
      /label=EGFP_N_primer
      /ApEinfo_fwdcolor=#ffcc66
      /ApEinfo_revcolor=#ffcc66
      /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
      width 5 offset 0
misc_feature    5377..5430
      /label=dT2A
      /ApEinfo_fwdcolor=#fff83d
      /ApEinfo_revcolor=#fff83d
      /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
      width 5 offset 0
CDS             5443..6867
      /label=tdTomato
```

```

    /ApEinfo_fwdcolor=#fdcdcf
    /ApEinfo_revcolor=#cde7f7
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
gene      6761..6784
    /gene="dTomato (variant)"

/translation="MVSKGEEVIKEFMRFKVRMEGSMNGHEFEIEGEGEGRPYEGTQT
AKLKVTKGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYKKLSFPEGFKWERVMNFED
GGLVTVTQDSSLQDGLTIYKVKMRGTNFPDGPVMQKKTMGWEASTERLYPRDGVKLG
EIHQALKLDGGHYLVEFKTIYMAKKPVQLPGYYYVDTKLDITSHNEDYTIVEQYERS
EGRHHLFLGHGTGSTGSGSGTASSEDNNMAVIKEFMRFKVRMEGSMNGHEFEIEGEG
EGRPYEGTQAKLKVTKGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYKKLSFPEGF
KWERVMNFEDGGLVTVTQDSSLQDGLTIYKVKMRGTNFPDGPVMQKKTMGWEASTER
LYPRDGVKGEIHQALKLDGGHYLVEFKTIYMAKKPVQLPGYYYVDTKLDITSHNED
    YTIVEQYERSEGRHHLFLYGMDELYK*"
    /label=dTomato (variant)
    /ApEinfo_fwdcolor=#ffffcc
    /ApEinfo_revcolor=#ffffcc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
promoter  complement(3419..3447)
    /label=AmpR_promoter
    /ApEinfo_fwdcolor=#ccffed
    /ApEinfo_revcolor=#ccffed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature  6035..6058
```

```
/translation="MVSKGEEVIKEFMRFKVRMEGSMNGHEFEIEGEGEGRPYEGTQT  
AKLKVTKGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYKLSFPEGFKWERVMNFED  
GGLVTVTQDSSLQDGLTIYVKMRGTNFPDGPVMQKKTMGWEASTERLYPRDGLKGG  
EIHQALKLDGGHYLVEFKTIYMAKKPVQLPGYYYVDTKLDITSHNEDYTIVEQYERS  
EGRHHLFLGHGTGSTGSGSSGTASSEDNNMAVIKEFMRFKVRMEGSMNGHEFEIEGEG  
EGRPYEGTQAKLKVTKGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYKLSFPEGF  
KWERVMNFEDGGLVTVTQDSSLQDGLTIYVKMRGTNFPDGPVMQKKTMGWEASTER  
LYPRDGLKGGEIHQALKLDGGHYLVEFKTIYMAKKPVQLPGYYYVDTKLDITSHNED  
    YTIVEQYERSEGRHHLFLYGMDELYK*"  
    /label=dsRed1_C_primer  
    /ApEinfo_fwdcolor=#ff3600  
    /ApEinfo_revcolor=#ff3600  
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}  
width 5 offset 0  
gene      complement(2517..3377)  
    /gene="Ampicillin"  
    /label=Ampicillin  
    /ApEinfo_fwdcolor=#ffffcc  
    /ApEinfo_revcolor=#ffffcc  
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}  
width 5 offset 0  
misc_feature  6761..6784  
  
/translation="MVSKGEEVIKEFMRFKVRMEGSMNGHEFEIEGEGEGRPYEGTQT  
AKLKVTKGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYKLSFPEGFKWERVMNFED  
GGLVTVTQDSSLQDGLTIYVKMRGTNFPDGPVMQKKTMGWEASTERLYPRDGLKGG
```



```
EIQALKLKDGGHYLVEFKTIYMAKKPVQLPGYYYVDTKLDITSHNEDYTIVEQYERS
EGRHHLFLGHGTGSTGSGSSGTASSEDNNMAVIKEFMRFKVRMEGSMNGHEFEIEGEG
EGRPYEGTQTAKLKVTKGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYKKLSFPEGF
KWERMNFEDGGLVTVTQDSSLQDGLTIYKVKMRGTNFPDGPVMQKKTMGWEASTER
LYPRDGLKGEIQALKLKDGGHYLVEFKTIYMAKKPVQLPGYYYVDTKLDITSHNED
      YTIVEQYERSEGRHHLFLYGMDELYK*"
      /label=dsRed1_C_primer(1)
      /ApEinfo_label=dsRed1_C_primer
      /ApEinfo_fwdcolor=#ff3600
      /ApEinfo_revcolor=#ff3600
      /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
      width 5 offset 0
  rep_origin    complement(1743..2362)

/translation="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGY
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLTTIGGPKELTAFLHNMGDHVTRL
DRWEPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPL
LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
      EIGASLIKHW*"
      /label=pBR322_origin
      /ApEinfo_fwdcolor=pink
      /ApEinfo_revcolor=pink
      /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
      width 5 offset 0
  misc_feature  5304..5325

/translation="MVSKGEELFTGVVPIVELDGDVNGHKFSVSGEGEGDATYGKLT
```

```
LKFICTTGKLPVPWPTLVTTLTYGVCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFK

DDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNNSHNVYIMADKQKN

GIKVNFKIRHNIEDGSVQLADHYQNTPIGDGPVLLPDNHYLSTQSALS KDPNEKRDH
    MVLLEFVTAAGITLGMDELYKSGLSRAQASNSAVDGTAGPGSTGSR*"
    /label=EGFP_C_primer
    /ApEinfo_fwdcolor=#ffcc66
    /ApEinfo_revcolor=#ffcc66
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    4651..5367
    /label=EGFP(1)
    /ApEinfo_label=EGFP
    /ApEinfo_fwdcolor=#49ff00
    /ApEinfo_revcolor=#49ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    5308..5327
    /label=EGFP_F
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff00bd
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    complement(5300..5322)
    /label=EGFP_R2
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    complement(4772..4797)
    /label=EGFP_R3
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
```

```
width 5 offset 0
rep_origin    complement(1740..2422)
              /label=ColE1 origin
              /ApEinfo_fwdcolor=gray50
              /ApEinfo_revcolor=gray50
              /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind   4961..4982
              /label=nvGF
              /ApEinfo_fwdcolor=#ff00bd
              /ApEinfo_revcolor=#ff0003
              /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind   complement(5293..5312)
              /label=nvGR
              /ApEinfo_fwdcolor=#ff00bd
              /ApEinfo_revcolor=#ff0003
              /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind   6835..6853
              /label=tdTomato-Fwd
              /ApEinfo_fwdcolor=#ff00bd
              /ApEinfo_revcolor=#ff0003
              /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
CDS           complement(2520..3179)
              /label=AmpR
              /ApEinfo_fwdcolor=yellow
              /ApEinfo_revcolor=yellow
              /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind   complement(6179..6197)
              /label=tdTomato-Rev
              /ApEinfo_fwdcolor=#ff00bd
              /ApEinfo_revcolor=#ff0003
              /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
```

```
width 5 offset 0
primer_bind    complement(3098..3122)
               /label=Amp-GF
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind    2640..2663
               /label=Amp-GR1
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind    complement(7703..7722)
               /label=SEQ-EBV-rev(12A02)
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind    4780..4802
               /label=EGFP_F2
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind    6161..6182
               /label=tdLink primer
               /ApEinfo_fwdcolor=#ff00bd
               /ApEinfo_revcolor=#ff0003
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
CDS            6964..7608
               /label=Rab11
               /ApEinfo_fwdcolor=#99ccff
               /ApEinfo_revcolor=#cde7f7
               /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
```

```
width 5 offset 0
promoter complement(1405..1434)

/translation="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGY
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLTTIGGPKELTAFLHNMGDHVTRL
DRWEPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPL
LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
EIGASLIKHW*"
/label=lac_promoter
/ApEinfo_fwdcolor=#ccffed
/ApEinfo_revcolor=#ccffed
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature complement(1369..1391)

/translation="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGY
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLTTIGGPKELTAFLHNMGDHVTRL
DRWEPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPL
LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
EIGASLIKHW*"
/label=M13_pUC_rev_primer
/ApEinfo_fwdcolor=#ff3600
/ApEinfo_revcolor=#ff3600
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
promoter complement(1352..1370)
```

```
/translation="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGY
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHVTRL
DRWEPELNEAIPNDERDTMPVAMATTLRKLTTGELLTLASRQQLIDWMEADKVAGPL
LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
    EIGASLIKHW*"
    /label=M13_reverse_primer
    /ApEinfo_fwdcolor=#ccffed
    /ApEinfo_revcolor=#ccffed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
gene    complement(143..742)
        /gene="puro (variant)"

/translation="MGSCAPFGRALRVVGRASGTGLAGHAPGARSFGHLDVGGDGEAE
PLVEGEVAGRGGLEGGHPGALGRLHSGEHDGAAQTLALVVRDADGGQEPRGLLGPV
RRQEAFLHLLLRGQPGTAQLGHARADLGEHRPRFDALRRGPDRHRGAVVRDPHLADVEP
DAREEEFLQLGDPLDVAVRVDGVARGGVVGERGGEGAYGPGDVVAGGEAHRGLVLGHG
    RSSPCEGSGAWVRGWRHRSWRPTCRHASRYRIPAARGICCNL*"
    /label=puro (variant)
    /ApEinfo_fwdcolor=#ffffcc
    /ApEinfo_revcolor=#ffffcc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    complement(843..1201)
                /label=Copia Promoter
                /ApEinfo_fwdcolor=#cde7f7
                /ApEinfo_revcolor=#cde7f7
```

```
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    complement(1352..1372)
    /label=M13-rev
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=green
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_binding   complement(1378..1400)
    /label=Lac0
    /ApEinfo_fwdcolor=#6495ed
    /ApEinfo_revcolor=#6495ed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    complement(1352..1372)
    /label=M13R_GW
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    1230..1252
    /label=Seq-EB-R
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    1293..1318
    /label=CMV-R-EB-N
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
misc_feature   complement(3820..3975)
    /label=lacZ_a
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
```

```
promoter      /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
              width 5 offset 0
              3956..3972
              /label=M13_forward20_primer
              /ApEinfo_fwdcolor=#ccffed
              /ApEinfo_revcolor=#ccffed
              /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
              width 5 offset 0
misc_feature  3941..3963
              /label=M13_pUC_fwd_primer
              /ApEinfo_fwdcolor=#ff3600
              /ApEinfo_revcolor=#ff3600
              /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
              width 5 offset 0
misc_feature  complement(3606..3628)
              /label=pGEX_3_primer
              /ApEinfo_fwdcolor=#ff3600
              /ApEinfo_revcolor=#ff3600
              /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
              width 5 offset 0
misc_feature  3982..4349
              /label=MT-promoter
              /ApEinfo_fwdcolor=cyan
              /ApEinfo_revcolor=#00ff00
              /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
              width 5 offset 0
misc_feature  4385..4402
              /label=Metallothionein_primer
              /ApEinfo_fwdcolor=#ff3600
              /ApEinfo_revcolor=#ff3600
              /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
              width 5 offset 0
primer_bind   3955..3972
              /label=M13-fwd
              /ApEinfo_fwdcolor=cyan
              /ApEinfo_revcolor=green
```



```

    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
CDS      complement(3816..3884)
        /label=LacZ alpha
        /ApEinfo_fwdcolor=#6495ed
        /ApEinfo_revcolor=#6495ed
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind 3929..3952
        /label=M13F
        /ApEinfo_fwdcolor=#0a00ff
        /ApEinfo_revcolor=#0a00ff
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind 3955..3972
        /label=M13F_GW
        /ApEinfo_fwdcolor=#ff00bd
        /ApEinfo_revcolor=#ff0003
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind 3491..3508
        /label=pQE60-F
        /ApEinfo_fwdcolor=#ff00bd
        /ApEinfo_revcolor=#ff0003
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind 4325..4342
        /label=SEQ-MT-F2
        /ApEinfo_fwdcolor=#ff00bd
        /ApEinfo_revcolor=#ff0003
        /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
ORIGIN
1 ataagctgca ataaacaagt tctagagtcg gtgggcctcg ggggcgggtg cggggtcggc
61 ggggccgcc cgggtggctt cggtcggagc catggggtcg tgcgctcctt tcggtcgggc
121 gctgcgggtc gtggggcggg cgtcaggcac cgggcttgcg ggtcatgcac caggtgcgcg
```

```
181 gtccttcggg cacctcgacg tcggcgggta cggggaagcc gagccgctcg tagaagggga
241 ggttgcgggg cgcggaggtc tccaggaagg cgggcacccc ggcgcgctcg gccgcctcca
301 ctccggggag cacgacggcg ctgcccagac ctttgccctg gtggtcgggc gagacgccga
361 cgggtggccag gaaccacgcg ggctccttgg gccgggtgcgg cgccaggagg ctttccatct
421 gttgctgcgc ggccagccgg gaaccgctca actcggccat gcgcggggcg atctcggcga
481 acaccgcccc cgcttcgacg ctctccggcg tgggccagac cgccaccgcg gcgccgtcgt
541 ccgcgacca caccttgccg atgtcgagcc cgacgcgcgt gaggaagagt tcttcagcgt
601 cggtgacccg ctcgatgtgg cgggtccgggt cgacgggtgt gcgcgtggcg gggtagtcgg
661 cgaacgcggc ggcgaggggt cgtacggccc gggggacgtc gtcgcgggtg gcgaggcgca
721 ccgtgggctt gtactcggtc atggaaggtc gtctccttgt gaggggtcag gggcgtgggt
781 caggggatgg tggcggcacc ggtcgtggcg gccgacctg aggcatgcaa gctatcgaat
841 tcctgcagcc cgggggatct gttgtaattt ataatttata tttcctttct taataataa
901 ataaatagtc aagtttatgt ttgagtttta tgatttata ttttaagttat ttcaactgca
961 acaccagcac cacgacctac ttacagcaaa aaacgtacaa gaaggaaaga aggaataaaa
1021 agagtgggat tctcttacia tatgttttat ggcataaaag gtgtggccat tcatatcaaa
1081 tataaagtag tgttgtttaa cgttactttt gtaggttgaa tagtatattc caacagatga
1141 tgaggggttc ccaatcctaa acccatttgc cgttcccaga agcatgaaac caccacgcac
1201 cggatcctct agaacaacia caattgcatt ctttttatgt ttcaggttca gggggagggtg
1261 tgggaggttt tttaaagcaa gtaaacctc taaaaatgtg gtatggctga ttatgatcag
1321 tcgacctgca ggcattgcaag cttggcgtaa tcatggcat agctgtttcc tgtgtgaaat
1381 tgttatccgc tcacaattcc acacaacata cgagccggaa gcataaagt taaagcctgg
1441 ggtgcctaata gagtgagcta actcacatta attgcgttgc gctcactgcc cgctttccag
1501 tcgggaaacc tgcctgcca gctgcattaa tgaatcggc aacgcgcggg gagaggcggg
1561 ttgcgtattg ggcGCTCTTC cgcttcctcg ctactgact cgctgcgctc ggtcgttcgg
1621 ctgcggcgag cggtatcagc tcaactcaaag gcggtaatac ggttatccac agaatcaggg
1681 gataacgcag gaaagaacat gtgagcaaaa ggccagcaaa aggccaggaa ccgtaaaaag
1741 gccgcgttgc tggcgttttt ccataggctc cgccccctg acgagcatca caaaaatcga
1801 cgctcaagtc agaggtggcg aaacccgaca ggactataaa gataaccaggc gtttccccct
1861 ggaagctccc tcgtgcgctc tcctgttccg accctgccg ttaccggata cctgtccgcc
1921 tttctccctt cgggaagcgt ggcgctttct catagctcac gctgtaggta tctcagttcg
1981 gtgtaggtcg ttcgctcaa gctgggctgt gtgcacgaac cccccgtta gcccgaccgc
2041 tgcgccttat ccggtacta tcgtcttgag tccaacccgg taagacacga cttatcgcca
2101 ctggcagcag ccaactgtaa caggattagc agagcgagg atgtaggcgg tgctacagag
2161 ttcttgaagt ggtggcctaa ctacggctac actagaagaa cagtatttgg tatctgcgct
2221 ctgctgaagc cagttacctt cggaaaaaga gttggtagct cttgatccgg caaacaacc
2281 accgctggta gcgggtggtt ttttgtttgc aagcagcaga ttacgcgcag aaaaaaagga
```

2341 tctcaagaag atcctttgat cttttctacg gggctgacg ctcaagtggaa cgaaaactca  
2401 cgtaaggga ttttggtcat gagattatca aaaaggatct tcacctagat ctttttaaat  
2461 taaaaatgaa gttttaaatc aatctaaagt atatatgagt aaacttggtc tgacagttac  
2521 caatgcttaa tcagtgaggc acctatctca gcgatctgtc tatttcgttc atccatagtt  
2581 gcctgactcc ccgtcgtgta gataactacg atacgggagg gcttaccatc tggccccagt  
2641 gctgcaatga taccgcgaga cccacgctca ccggctccag atttatcagc aataaaccag  
2701 ccagccggaa gggccgagcg cagaagtggc cctgcaactt tatccgcctc catccagtct  
2761 attaatgtt gccgggaagc tagagtaagt agttcgccag ttaatagttt gcgcaacggt  
2821 gttgccattg ctacaggcat cgtggtgtca cgctcgtcgt ttggtatggc ttcattcagc  
2881 tccggttccc aacgatcaag gcgagttaca tgatcccca tgttgtgcaa aaaagcgggt  
2941 agctccttcg gtcctccgat cgttgtcaga agtaagtgg ccgcagtgtt atcactcatg  
3001 gttatggcag cactgcataa ttctcttact gtcatgcat ccgtaagatg cttttctgtg  
3061 actggtgagt actcaaccaa gtcattctga gaatagtgtg tgcggcgacc gagttgctct  
3121 tgcccggcgt caatacggga taataccgcg ccacatagca gaactttaa agtgctcatc  
3181 attggaaaac gttcttcggg gcgaaaactc tcaaggatct taccgctgtt gagatccagt  
3241 tcgatgtaac ccactcgtgc acccaactga tcttcagcat cttttacttt caccagcgtt  
3301 tctgggtgag caaaaacagg aaggcaaat gccgcaaaa agggaataag ggcgacacgg  
3361 aatgttgaa tactcatact cttcctttt caatattatt gaagcattta tcagggttat  
3421 tgtctcatga gcggatacat atttgaatgt atttagaaa ataaacaaat aggggttccg  
3481 gcacatttc cccgaaaagt gccacctgac gtctaagaaa ccattattat catgacatta  
3541 acctataaaa ataggcgtat cacgaggccc tttcgtctcg cgcgtttcgg tgatgacggt  
3601 gaaaacctct gacacatgca gctcccggag acggtcacag cttgtctgta agcggatgcc  
3661 gggagcagac aagcccgtca gggcgcgtca gcgggtgttg gcgggtgtcg gggctggctt  
3721 aactatgcgg catcagagca gattgtactg agagtgcacc atatgcggtg tgaataaccg  
3781 cacagatgcg taaggagaaa ataccgcatc aggcgccatt cgccattcag gctgcgcaac  
3841 tgttgggaag ggcgatcggc gcgggcctct tcgctattac gccagctggc gaaaggggga  
3901 tgtgctgcaa ggcgattaag ttgggtaacg ccagggtttt cccagtcacg acgttgtaaa  
3961 acgacggcca gtgaattaat tcgttgcagg acaggatgtg gtgcccgatg tgactagctc  
4021 tttgctgca gccgtcctat cctctggttc cgataagaga cccagaactc cggccccca  
4081 ccgcccaccg ccacccccat acatatgtgg tacgcaagta agagtgcctg cgcagcccc  
4141 atgtgcccc ccaagagctt tgcattccat acaagtcccc aaagtggaga accgaaccaa  
4201 ttcttcgagg gcagaacaaa agcttctgca cacgtctcca ctgaatttg gagccggcgg  
4261 gcgtgtgcaa aagaggtgaa tcgaacgaaa gaccctgtg taaagccgcg tttccaaaat  
4321 gtataaaacc gagagcatct ggccaatgtg catcagttgt ggtcagcagc aaaatcaagt  
4381 gaatcatctc agtgcaacta aaggggggat ctagatcggg gtaccaccAT GAGGCTTCGG  
4441 GAGCCGCTCC TGAGCGGCAG CGCCGCGATG CCAGGCGCGT CCCTACAGCG GGCCTGCCG

4501 CTGCTCGTGG CCGTCTGCGC TCTGCACCTT GGCCTCACCC TCGTTTACTA CCTGGCTGGC  
4561 GCAGACCTGA GCCGCCTGCC CCAACTGGTC GGAGTCTCCA CACCGCTGCA GTCGACGGTA  
4621 CCGCGGGCCC GGGATCCACC GGTcgccacc ATGGTGAGCA AGGGCGAGGA GCTGTTACC  
4681 GGGGTGGTGC CCATCCTGGT CGAGCTGGAC GGCAGCTAA ACGGCCACAA GTTCAGCGTG  
4741 TCCGGCGAGG GCGAGGGCGA TGCCACCTAC GGCAAGCTGA CCCTGAAGTT CATCTGCACC  
4801 ACCGGCAAGC TGCCCCTGCC CTGGCCCACC CTCGTGACCA CCCTGACCTA CGGCGTGCAG  
4861 TGCTTCAGCC GCTACCCCGA CCACATGAAG CAGCACGACT TCTTCAAGTC CGCCATGCCC  
4921 GAAGGCTACG TCCAGGAGCG CACCATCTTC TTCAAGGACG ACGGCAACTA CAAGACCCGC  
4981 GCCGAGGTGA AGTTCGAGGG CGACACCCTG GTGAACCGCA TCGAGCTGAA GGGCATCGAC  
5041 TTCAAGGAGG ACGGCAACAT CCTGGGGCAC AAGCTGGAGT ACAACTACAA CAGCCACAAC  
5101 GTCTATATCA TGGCCGACAA GCAGAAGAAC GGCATCAAGG TGAACTTCAA GATCCGCCAC  
5161 AACATCGAGG ACGGCAGCGT GCAGCTCGCC GACCACTACC AGCAGAACAC CCCCATCGGC  
5221 GACGGCCCCG TGCTGCTGCC CGACAACCAC TACCTGAGCA CCCAGTCCGC CTGAGCAAAA  
5281 GACCCCAACG AGAAGCGCGA TCACATGGTC CTGCTGGAGT TCGTGACCGC CGCCGGGATC  
5341 ACTCTCGGCA TGGACGAGCT GTACAAGgga ggtggaGAAG GACGCGGAG CCTACTGACT  
5401 TCGGAGATG TCGAAGAGAA CCCTGGCCCT ggtGccaccA TGGTGAGCAA GGGCGAGGAG  
5461 GTCATCAAAG AGTTCATGCG CTTCAAGGTG CGCATGGAGG GCTCCATGAA CGGCCACGAG  
5521 TTCGAGATCG AGGGCGAGGG CGAGGGCCGC CCCTACGAGG GCACCCAGAC CGCCAAGCTG  
5581 AAGGTGACCA AGGGCGGCC CCTGCCCTTC GCCTGGGACA TCCTGTCCCC CCAGTTCATG  
5641 TACGGCTCCA AGGCGTACGT GAAGCACCCC GCCGACATCC CCGATTACAA GAAGCTGTCC  
5701 TTCCCCGAGG GCTTCAAGTG GGAGCGCGTG ATGAACTTCG AGGACGGCGG TCTGGTGACC  
5761 GTGACCCAGG ACTCCTCCCT GCAGGACGGC ACGCTGATCT ACAAGGTGAA GATGCGCGGC  
5821 ACCAACTTCC CCCCCGACGG CCCCATAATG CAGAAGAAGA CCATGGGCTG GGAGGCCTCC  
5881 ACCGAGCGCC TGTACCCCGC CGACGGCGTG CTGAAGGGCG AGATCCACCA GGCCCTGAAG  
5941 CTGAAGGACG GCGGCCACTA CCTGGTGGAG TTCAAGACCA TCTACATGGC CAAGAAGCCC  
6001 GTGCAACTGC CCGGCTACTA CTACGTGGAC ACCAAGCTGG ACATCACCTC CCACAACGAG  
6061 GACTACACCA TCGTGGAACA GTACGAGCGC TCCGAGGGCC GCCACCACCT GTTCCTGGGG  
6121 CATGGCACCG GCAGCACCGG CAGCGGCAGC TCCGGCACCG CCTCCTCCGA GGACAACAAC  
6181 ATGGCCGTC TCAAAGAGTT CATGCGCTTC AAGGTGCGCA TGGAGGGCTC CATGAACGGC  
6241 CACGAGTTCC AGATCGAGGG CGAGGGCGAG GGCCGCCCCT ACGAGGGCAC CCAGACCGCC  
6301 AAGCTGAAGG TGACCAAGGG CGGCCCCCTG CCCTTCGCTT GGGACATCCT GTCCCCCAG  
6361 TTCATGTACG GCTCCAAGGC GTACGTGAAG CACCCCGCCG ACATCCCCGA TTACAAGAAG  
6421 CTGTCCTTCC CCGAGGGCTT CAAGTGGGAG CGCGTGATGA ACTTCGAGGA CGGCGGTCTG  
6481 GTGACCGTGA CCCAGGACTC CTCCCTGCAG GACGGCACGC TGATCTACAA GGTGAAGATG  
6541 CGCGGCACCA ACTTCCCCC CGACGGCCCC GTAATGCAGA AGAAGACCAT GGGCTGGGAG  
6601 GCCTCCACCG AGCGCCTGTA CCCCCGCGAC GGCCTGCTGA AGGGCGAGAT CCACCAGGCC

```

6661 CTGAAGCTGA AGGACGGCGG CCACTACCTG GTGGAGTTCA AGACCATCTA CATGGCCAAG
6721 AAGCCCGTGC AACTGCCCGG CTACTACTAC GTGGACACCA AGCTGGACAT CACCTCCCAC
6781 AACGAGGACT ACACCATCGT GGAACAGTAC GAGCGCTCCG AGGGCCGCCA CCACCTGTTC
6841 CTGTACGGCA TGGACGAGCT GTACAAGTCC GGACTCAGAT CTCGAGCTCA AGCTTCGAAT
6901 TCTGCAGTCG ACGGTACCGC GGGCCCCGGA TCATCAACAA GTTTGTACAA AAAAGCAGGC
6961 TTAATGGGTG CAAGAGAAGA CGAGTACGAT TATCTGTTCA AAGTTGTCNT TATCGGTGAC
7021 TCCGGTGTG GCAAAAAGTAA TTTGCTCTCA CGTTTCACGC GCAATGAATT CAACTTGAG
7081 TCCAAGTCGA CGATTGGCGT TGAGTTTGCA ACGCGCAGCA TAGAGGTCGA TGGCAAAAACA
7141 ATTAAAGCGC AAATCTGGGA TACGGCCGGC CAGGAGCGTT ATCGGCCCAT CACCTCTGCC
7201 TACTACCGCG GTGCCGTGGG GGCCCTGCTC GTCTATGACA TTGCCAAGCA TCTGACCTAC
7261 GAGAACGTGG AGCGGTGGCT GCGGGAATTG CGCGACCATG CCGACCAGAA CATCGTCATC
7321 ATGCTGGTGG GCAACAAGTC CGACTTGGCG CACTTGGCGT CCGTGCCAC GGACGAGGCG
7381 AAGCTGTTTG CCGAGCGCAA CGGCTTGAGT TTCATAGAAA CCTCGGCCCT CGACTCAACG
7441 AACGTTGAAA CGGCATTCCA GAACATACTC ACAGAGATCT ATCGCATTGT GTCGCAGAAA
7501 CAGATCAGAG ATCCGCCGGA AGGCGACGTC ATCCGCCCGT CGAACGTGGA GCCCATCGAC
7561 GTAAAGCCGA CTGTCACCGC CGATGTGCGC AAACAGTGCT GTCAGTAACG cgtaccggtC
7621 ATCATACCA TCACCATTGA gtttaaacc gctgatcagc ctcgactgtg ctttctaaga
7681 tccagacatg ataagataca ttgatgagtt tggacaaacc acaactagaa tgcagtgaaa
7741 aaaatgcttt atttgtgaaa tttgtgatgc tattgcttta tttgtaacca tt

```

//

pMT-GalT-EGFP-T2A-tdTomato-Rab6

```

LOCUS      pMT_GalT_GFP_T2A          7705 bp ds-DNA      circular      28-FEB-
2020
DEFINITION pMT-puro Sequencing Result
ORGANISM   other sequences; artificial sequences; vectors.
COMMENT    pMT-GalT-EGFP-T2A-tdTom-Vamp3 from 1 to 7513
COMMENT    pMT-EGFP-hVamp3 from 1 to 5841
COMMENT    pMT-puro from 1 to 4724
COMMENT    ApEinfo:methylated:1
FEATURES   Location/Qualifiers
misc_feature complement(7616..7635)
            /label=EBV_rev_primer
            /ApEinfo_fwdcolor=#ff3600
            /ApEinfo_revcolor=#ff3600
            /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
            width 5 offset 0

```

```
misc_feature    4429..4605
                /label=GaT
                /ApEinfo_fwdcolor=cyan
                /ApEinfo_revcolor=#00ff00
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
CDS             7496..7519
                /locus_tag="Rab6"
                /label=Rab6
                /ApEinfo_fwdcolor=#99ccff
                /ApEinfo_revcolor=#cde7f7
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
misc_feature    complement(7573..7590)
                /label=BGH_rev_primer
                /ApEinfo_fwdcolor=#ff3600
                /ApEinfo_revcolor=#ff3600
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
gene            4654..5367
                /gene="EGFP"

/translacion="MVSKGEELFTGVVPIVELDGDVNGHKFSVSGEGEGDATYGKLT
LKFICTTGKLPVPWPTLVTTLTYGVCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFK
DDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKN
GIKVNFKIRHNIEDGSVQLADHYQNTPIGDGPVLLPDNHYLSTQSALS KDPNEKRDH
MVLLEFVTAAGITLGMDELYKSGLRSAQASNSAVDGTAGPGSTGSR*"
                /label=EGFP
                /ApEinfo_fwdcolor=#66ff66
                /ApEinfo_revcolor=#ffffcc
                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                width 5 offset 0
misc_feature    complement(4696..4717)
```

```
/translation="MVSKGEELFTGVVPIVELDGDVNGHKFSVSGEGEDATYGKLT
LKFICTTGKLPVPWPTLVTTLTYGVCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFK
DDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNNSHNVYIMADKQKN
GIKVNFKIRHNIEDGSVQLADHYQNTPIGDGPVLLPDNHYLSTQSALS KDPNEKRDH
      MVLLEFVTAAGITLGMDELYKSGLRSAQASNSAVDGTAGPGSTGSR*"
      /label=EGFP_N_primer
      /ApEinfo_fwdcolor=#ffcc66
      /ApEinfo_revcolor=#ffcc66
      /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
CDS      6898..7495
      /locus_tag="Rab6(1)"
      /label=Rab6(1)
      /ApEinfo_label=Rab6
      /ApEinfo_fwdcolor=#99ccff
      /ApEinfo_revcolor=#cde7f7
      /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
misc_feature 5377..5430
      /label=dT2A
      /ApEinfo_fwdcolor=#fff83d
      /ApEinfo_revcolor=#fff83d
      /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
CDS      5443..6867
      /label=tdTomato
      /ApEinfo_fwdcolor=#fdcdcf
      /ApEinfo_revcolor=#cde7f7
      /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
gene      6761..6784
      /gene="dTomato (variant)"
```

```
/translation="MVSKGEEVIKEFMRFKVRMEGSMNGHEFEIEGEGEGRPYEGTQT  
AKLKVTGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYKKLSFPEGFKWERVMNFED  
GGLVTVTQDSSLQDGLTIYVKMRGTNFPDGPVMQKKTMGWEASTERLYPRDGLKGG  
EIHQALKLDGGHYLVEFKTIYMAKKPVQLPGYYYVDTKLDITSHNEDYTIVEQYERS  
EGRHHLFLGHGTGSTGSGSSGTASSEDNNMAVIKEFMRFKVRMEGSMNGHEFEIEGEG  
EGRPYEGTQAKLKVTGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYKKLSFPEGF  
KWERVMNFEDGGLVTVTQDSSLQDGLTIYVKMRGTNFPDGPVMQKKTMGWEASTER  
LYPRDGLKGEIHQALKLDGGHYLVEFKTIYMAKKPVQLPGYYYVDTKLDITSHNED  
YTIVEQYERSEGRHHLFLYGMDELYK*"  
/label=dTomato (variant)  
/ApEinfo_fwdcolor=#ffffcc  
/ApEinfo_revcolor=#ffffcc  
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}  
width 5 offset 0  
misc_feature 6868..6873  
/locus_tag="SpeI"  
/label=SpeI  
/ApEinfo_fwdcolor=#ffffcc  
/ApEinfo_revcolor=#ffffcc  
/ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}  
width 5 offset 0  
misc_feature 6035..6058  
  
/translation="MVSKGEEVIKEFMRFKVRMEGSMNGHEFEIEGEGEGRPYEGTQT  
AKLKVTGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYKKLSFPEGFKWERVMNFED  
GGLVTVTQDSSLQDGLTIYVKMRGTNFPDGPVMQKKTMGWEASTERLYPRDGLKGG
```



```
EIHQALKLKDGGHYLVEFKTIYMAKKPVQLPGYYYVDTKLDITSHNEDYTIVEQYERS
EGRHHLFLGHGTGSTGSGSSGTASSEDNNMAVIKEFMRFKVRMEGSMNGHEFEIEGEG
EGRPYEGTQTAKLKVTKGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYKKLSFPEGF
KWERMVNFEDGGLVTVTQDSSLQDGLTIYKVKMRGTNFPDGPVMQKKTMGWEASTER
LYPRDGLKGEIHQALKLKDGGHYLVEFKTIYMAKKPVQLPGYYYVDTKLDITSHNED
    YTIVEQYERSEGRHHLFLYGMDELYK*"
    /label=dsRed1_C_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    7105..7107
    /locus_tag="Q71L_CAG>CTG"
    /label=Q71
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    6761..6784

/translation="MVSKGEEVIKEFMRFKVRMEGSMNGHEFEIEGEGEGRPYEGTQT
AKLKVTKGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYKKLSFPEGFKWERMVNFED
GGLVTVTQDSSLQDGLTIYKVKMRGTNFPDGPVMQKKTMGWEASTERLYPRDGLKGE
EIHQALKLKDGGHYLVEFKTIYMAKKPVQLPGYYYVDTKLDITSHNEDYTIVEQYERS
EGRHHLFLGHGTGSTGSGSSGTASSEDNNMAVIKEFMRFKVRMEGSMNGHEFEIEGEG
EGRPYEGTQTAKLKVTKGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYKKLSFPEGF
```

```
KWERVMNFEDGGLVTVTQDSSLQDGLIYKVKMRGTNFPDGPVMQKKTMGWEASTER

LYPRDGLKGEIHQALKLKDGGHYLVEFKTIYMAKKPVQLPGYYYYVDTKLDITSHNED
    YTIVEQYERSEGRHHLFLYGMDELYK*"
    /label=dsRed1_C_primer(1)
    /ApEinfo_label=dsRed1_C_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    5304..5325

/translation="MVSKGEELFTGVVPIVELDGDVNGHKFSVSGEGEGDATYGKLT

LKFICTTGKLPVPWPTLVTTLTYGVCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFK

DDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNNSHNVYIMADKQKN

GIKVNFKIRHNIEDGSVQLADHYQNTPIGDGPVLLPDNHYLSTQSALS KDPNEKRDH
    MVLLEFVTAAGITLGMDELYKSGLRSAQASNSAVDGTAGPGSTGSR*"
    /label=EGFP_C_primer
    /ApEinfo_fwdcolor=#ffcc66
    /ApEinfo_revcolor=#ffcc66
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    5308..5327
    /label=EGFP_F
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff00bd
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    complement(5300..5322)
    /label=EGFP_R2
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
```

```
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    complement(7183..7202)
    /locus_tag="DRSC25636-R"
    /label=DRSC25636-R
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    complement(4772..4797)
    /label=EGFP_R3
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    4961..4982
    /label=nvGF
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    complement(5293..5312)
    /label=nvGR
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    6835..6853
    /label=tdTomato-Fwd
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
    width 5 offset 0
primer_bind    complement(6179..6197)
    /label=tdTomato-Rev
    /ApEinfo_fwdcolor=#ff00bd
```

```

    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature    6874..6897
    /label=Linker GL3
    /ApEinfo_fwdcolor=#ffcc66
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind    complement(7616..7635)
    /label=SEQ-EBV-rev(12A02)
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind    4780..4802
    /label=EGFP_F2
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
primer_bind    6161..6182
    /label=tdLink primer
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature    6984..6984
    /label=g>a
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature    6987..6987
    /label=c>a
    /ApEinfo_fwdcolor=cyan
```

```

    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature    7032..7032
    /label=a>g
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature    6970..6972
    /locus_tag="T26N ACC>AAC"
    /label=T26
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature    7519..7521
    /label=STOP
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature    complement(3820..3975)
    /label=lacZ_a
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
promoter        3956..3972
    /label=M13_forward20_primer
    /ApEinfo_fwdcolor=#ccffed
    /ApEinfo_revcolor=#ccffed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0
misc_feature    3941..3963
    /label=M13_pUC_fwd_primer
```

```

    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    complement(3606..3628)
    /label=pGEX_3_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
promoter        complement(3419..3447)
    /label=AmpR_promoter
    /ApEinfo_fwdcolor=#ccffed
    /ApEinfo_revcolor=#ccffed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
gene            complement(2517..3377)
    /gene="Ampicillin"
    /label=Ampicillin
    /ApEinfo_fwdcolor=#ffffcc
    /ApEinfo_revcolor=#ffffcc
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
rep_origin      complement(1743..2362)

/translation="MSIQHFRVALIPFFAAFLP VF AHPETLVKVKDAEDQLGARVGY
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHVTRL
DRWEPELNEAIPNDERDTMPVAMATTLRKLTTGELLTLASRQQLIDWMEADKVAGPL
LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
EIGASLIKHW*"
    /label=pBR322_origin
```

```

    /ApEinfo_fwdcolor=pink
    /ApEinfo_revcolor=pink
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
promoter    complement(1405..1434)

/translation="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGY
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHVTRL
DRWEPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPL
LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
    EIGASLIKHW*"
    /label=lac_promoter
    /ApEinfo_fwdcolor=#ccffed
    /ApEinfo_revcolor=#ccffed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    complement(1369..1391)

/translation="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGY
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHVTRL
DRWEPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPL
LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
    EIGASLIKHW*"
    /label=M13_pUC_rev_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
```

```

                                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                                width 5 offset 0
promoter      complement(1352..1370)

/translacion="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGY
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRRIHYSQNDLVE
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHVTRL
DRWEPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPL
LRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIA
                                EIGASLIKHW*"
                                /label=M13_reverse_primer
                                /ApEinfo_fwdcolor=#ccffed
                                /ApEinfo_revcolor=#ccffed
                                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                                width 5 offset 0
gene          complement(143..742)
                                /gene="puro (variant)"

/translacion="MGSCAPFGRALRVVGRASGTGLAGHAPGARSFGHLDVGGDGEAE
PLVEGEVAGRGGLEGGHPGALGRLHSGEHDGAAQTLALVVGRDADGGQEPRGLLGPV
RRQEFHLLLRGQPGAQLGHARADLGEHRPRFDALRRGPDHRGAVVRDPHLADVEP
DAREEEFLQLGDPLDVAVRVDGVARGGVVGERGGEGAYGPGDVVAGGEAHRGLVLGHG
                                RSPCEGSGAWVRGWWRHRSWRPTCRHASYRIPAARGICCNL*"
                                /label=puro (variant)
                                /ApEinfo_fwdcolor=#ffffcc
                                /ApEinfo_revcolor=#ffffcc
                                /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                                width 5 offset 0
misc_feature  3982..4349
```



```

    /label=MT-promoter
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=#00ff00
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    complement(843..1201)
    /label=Copia Promoter
    /ApEinfo_fwdcolor=#cde7f7
    /ApEinfo_revcolor=#cde7f7
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_feature    4385..4402
    /label=Metallothionein_primer
    /ApEinfo_fwdcolor=#ff3600
    /ApEinfo_revcolor=#ff3600
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    3955..3972
    /label=M13-fwd
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=green
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    complement(1352..1372)
    /label=M13-rev
    /ApEinfo_fwdcolor=cyan
    /ApEinfo_revcolor=green
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
rep_origin    complement(1740..2422)
    /label=ColE1 origin
    /ApEinfo_fwdcolor=gray50
    /ApEinfo_revcolor=gray50
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
CDS            complement(3816..3884)
```

```

    /label=LacZ alpha
    /ApEinfo_fwdcolor=#6495ed
    /ApEinfo_revcolor=#6495ed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
misc_binding    complement(1378..1400)
    /label=Lac0
    /ApEinfo_fwdcolor=#6495ed
    /ApEinfo_revcolor=#6495ed
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
CDS
    complement(2520..3179)
    /label=AmpR
    /ApEinfo_fwdcolor=yellow
    /ApEinfo_revcolor=yellow
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    3929..3952
    /label=M13F
    /ApEinfo_fwdcolor=#0a00ff
    /ApEinfo_revcolor=#0a00ff
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    3955..3972
    /label=M13F_GW
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    complement(1352..1372)
    /label=M13R_GW
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
    width 5 offset 0
primer_bind    3491..3508
```

```
    /label=pQE60-F
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    complement(3098..3122)
    /label=Amp-GF
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    2640..2663
    /label=Amp-GR1
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    4325..4342
    /label=SEQ-MT-F2
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    1230..1252
    /label=Seq-EB-R
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
primer_bind    1293..1318
    /label=CMV-R-EB-N
    /ApEinfo_fwdcolor=#ff00bd
    /ApEinfo_revcolor=#ff0003
    /ApEinfo_graphicformat=arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0
```

ORIGIN

```
1 ataagctgca ataaacaagt tctagagtcg gtgggcctcg ggggcgggtg cggggtcggc
61 ggggccgccc cgggtggctt cggtcggagc catggggtcg tgcgctcctt tcggtcgggc
121 gctgcgggtc gtggggcggg cgtcaggcac cgggcttgcg ggtcatgcac cagggtgcgcg
181 gtccttcggg cacctcgacg tcggcgggtga cggtgaagcc gagccgctcg tagaagggga
241 ggttgcgggg cgcgagggtc tccaggaagg cgggcacccc ggcgcgctcg gccgcctcca
301 ctccggggag cacgacggcg ctgcccagac cttgcccctg gtggtcgggc gagacgccga
361 cgggtggccag gaaccacgcg ggctccttgg gccggtgcgg cgccaggagg ctttccatct
421 gttgctgcgc ggccagccgg gaaccgctca actcggccat gcgcgggccc atctcggcga
481 acaccgccc cgcttcgacg ctctccggcg tggtcagac cgccaccgcg gcgccgtcgt
541 ccgcgacca caccttgccg atgtcgagcc cgacgcgcgt gaggaagagt tcttgagct
601 cggtgaccg ctcgatgtgg cggtcgggt cgacggtgtg gcgcgtggcg gggtagtcgg
661 cgaacgcggc ggcgagggtg cgtacggccc gggggacgtc gtcgcgggtg gcgaggcgca
721 ccgtgggctt gtactcggtc atggaaggtc gtctccttgt gaggggtcag gggcgtgggt
781 caggggatgg tggcggcacc ggtcgtggcg gccgacctg aggcattgca gctatcgaat
841 tcctgcagcc cgggggatct gttgtaattt ataatttata tttccttct taataataa
901 ataaatagtc aagtttatgt ttgagtttta tgatttatat ttttaagtat ttcaactgca
961 acaccagcac cacgacctac ttacagcaaa aaacgtacaa gaaggaaaga aggaataaaa
1021 agagtgggat tctcttacia tatgttttat ggcataaaag gtgtggccat tcatatcaaa
1081 tataaagtag tgttgtttaa cgttactttt gtaggttgaa tagtatattc caacagatga
1141 tgaggggttc ccaatcctaa acccatttgc cgttcccaga agcatgaaac caccacgcac
1201 cggatcctct agaacaacia caattgcatt ctttttatgt ttcaggttca gggggagggtg
1261 tgggagggtt tttaaagcaa gtaaacctc tacaatgtg gtatggctga ttatgatcag
1321 tcgacctgca ggcattgcaag cttggcgtaa tcatggcat agctgtttcc tgtgtgaaat
1381 tgttatccgc tcacaattcc acacaacata cgagccggaa gcataaagtg taaagcctgg
1441 ggtgccta at gagtgagcta actcacatta attgcgttgc gctcactgcc cgctttccag
1501 tcgggaaacc tgtcgtgcca gctgcattaa tgaatcggcc aacgcgcggg gagaggcgg
1561 ttgcgtattg ggcgctcttc cgcttctcgc ctactgact cgctgcgctc ggtcgttcgg
1621 ctgcggcgag cggtatcagc tcaactcaag gcggtaatat ggttatccac agaatcaggg
1681 gataacgcag gaaagaacat gtgagcaaaa ggccagcaaa aggccaggaa ccgtaaaaag
1741 gccgcgttgc tggcgttttt ccataggctc cgccccctg acgagcatca caaaaatcga
1801 cgctcaagtc agagggtggcg aaacccgaca ggactataaa gataccaggc gtttccccct
1861 ggaagctccc tcgtgcgctc tctgttccg accctgccgc ttaccggata cctgtccgcc
1921 tttctccctt cgggaagcgt ggcgctttct catagctcac gctgtaggta tctcagttcg
1981 gtgtaggtcg ttcgctcaa gctgggctgt gtgcacgaac cccccgtta gcccgaccgc
2041 tgcgccttat ccggtacta tcgtcttgag tccaacccgg taagacacga cttatcgcca
2101 ctggcagcag ccaactgtaa caggattagc agagcgaggt atgtaggcgg tgctacagag
```

2161 ttcttgaagt ggtggcctaa ctacggctac actagaagaa cagtatttgg tatctgcgct  
2221 ctgctgaagc cagttacctt cggaaaaaga gttggtagct cttgatccgg caaacaacc  
2281 accgctggta gcggtggttt ttttgtttgc aagcagcaga ttacgcgcag aaaaaagga  
2341 tctcaagaag atcctttgat cttttctacg gggcttgacg ctacgtggaa cgaaaactca  
2401 cgtaagga ttttggtcat gagattatca aaaaggatct tcacctagat ctttttaaat  
2461 taaaaatgaa gttttaaatc aatctaaagt atatatgagt aaacttggtc tgacagttac  
2521 caatgcttaa tcagtgaggc acctatctca gcgatctgtc tatttcgttc atccatagtt  
2581 gcctgactcc ccgtcgtgta gataactacg atacgggagg gcttaccatc tggccccagt  
2641 gctgcaatga taccgcgaga cccacgctca ccggctccag atttatcagc aataaaccag  
2701 ccagccgaa gggccgagcg cagaagtggc cctgcaactt tatccgcctc catccagtct  
2761 attaatgtt gccgggaagc tagagtaagt agttcgccag ttaatagttt gcgcaacggt  
2821 gttgccattg ctacaggcat cgtgggtgca cgctcgtcgt ttggtatggc ttcattcagc  
2881 tccggttccc aacgatcaag gcgagttaca tgatcccca tgttggtgcaa aaaagcgggt  
2941 agctccttcg gtcctccgat cgttgtcaga agtaagtgg ccgcagtgtt atcactcatg  
3001 gttatggcag cactgcataa ttctcttact gtcattgcat ccgtaagatg cttttctgtg  
3061 actggtgagt actcaaccaa gtcattctga gaatagtgta tgcggcgacc gagttgctct  
3121 tgcccggcgt caatacggga taataccgcg ccacatagca gaactttaa agtgctcatc  
3181 attgaaaaac gttcttcggg gcgaaaactc tcaaggatct taccgctgtt gagatccagt  
3241 tcgatgtaac ccactcgtgc acccaactga tcttcagcat cttttacttt caccagcgtt  
3301 tctgggtgag caaaaacagg aaggcaaaat gccgcaaaa agggaataag ggcgacacgg  
3361 aatggtgaa tactcatact cttccttttt caatattatt gaagcattta tcagggttat  
3421 tgtctcatga gcggatacat atttgaatgt atttagaaaa ataaacaaat aggggttccg  
3481 cgacacattc cccgaaaagt gccacctgac gtctaagaaa ccattattat catgacatta  
3541 acctataaaa ataggcgtat cacgaggccc tttcgtctcg cgcgtttcgg tgatgacggt  
3601 gaaaacctct gacacatgca gctcccggag acggtcacag cttgtctgta agcggatgcc  
3661 gggagcagac aagcccgtca gggcgcgtca gcgggtggtg gcgggtgctg gggctggctt  
3721 aactatgcgg catcagagca gattgtactg agagtgcacc atatgcggtg tgaataaccg  
3781 cacagatgcb taaggagaaa ataccgcatc aggcgccatt cgccattcag gctgcgcaac  
3841 tgttgggaag ggcgatcggc gcgggcctct tcgctattac gccagctggc gaaaggggga  
3901 tgtgctgcaa ggcgattaag ttgggtaacg ccagggtttt cccagtcacg acgttgtaaa  
3961 acgacggcca gtgaattaat tcgttgcagg acaggatgtg gtgcccgatg tgactagctc  
4021 tttgctgca gccgtcctat cctctggttc cgataagaga cccagaactc cggccccca  
4081 ccgcccaccg ccaccccat acatatgtgg tacgcaagta agagtgcctg cgcatgcccc  
4141 atgtgcccc ccaagagctt tgcattccat acaagtcccc aaagtggaga accgaaccaa  
4201 ttcttcgagg gcagaacaaa agcttctgca cacgtctcca ctcgaattg gagccggccg  
4261 gcgtgtgcaa aagaggtgaa tcgaacgaaa gaccctgtgt taaagccgcg tttccaaaat

4321 gtataaaacc gagagcatct ggccaatgtg catcagttgt ggtcagcagc aaaatcaagt  
4381 gaatcatctc agtgcaacta aaggggggat ctagatcggG GTACCaccAT GAGGCTTCGG  
4441 GAGCCGCTCC TGAGCGGCAG CGCCGCGATG CCAGGCGCGT CCCTACAGCG GGCCTGCCGC  
4501 CTGCTCGTGG CCGTCTGCGC TCTGCACCTT GGCCTCACCC TCGTTTACTA CCTGGCTGGC  
4561 CGCGACCTGA GCCGCCTGCC CCAACTGGTC GGAGTCTCCA CACCGCTGCA GTCGACGGTA  
4621 CCGCGGGCCC GGGATCCACC GGTcgccacc ATGGTGAGCA AGGGCGAGGA GCTGTTACC  
4681 GGGGTGGTGC CCATCCTGGT CGAGCTGGAC GGCACGTAA ACGGCCACAA GTTCAGCGTG  
4741 TCCGGCGAGG GCGAGGGCGA TGCCACCTAC GGCAAGCTGA CCCTGAAGTT CATCTGCACC  
4801 ACCGGCAAGC TGCCCGTGCC CTGGCCCACC CTCGTGACCA CCCTGACCTA CGGCGTGACG  
4861 TGCTTCAGCC GCTACCCCGA CCACATGAAG CAGCACGACT TCTTCAAGTC CGCCATGCCC  
4921 GAAGGCTACG TCCAGGAGCG CACCATCTTC TTCAAGGACG ACGGCAACTA CAAGACCCGC  
4981 GCCGAGGTGA AGTTCGAGGG CGACACCCTG GTGAACCGCA TCGAGCTGAA GGGCATCGAC  
5041 TTCAAGGAGG ACGGCAACAT CCTGGGGCAC AAGCTGGAGT ACAACTACAA CAGCCACAAC  
5101 GTCTATATCA TGGCCGACAA GCAGAAGAAC GGCATCAAGG TGAACTTCAA GATCCGCCAC  
5161 AACATCGAGG ACGGCAGCGT GCAGCTCGCC GACCACTACC AGCAGAACAC CCCCATCGGC  
5221 GACGGCCCCG TGCTGCTGCC CGACAACCAC TACCTGAGCA CCCAGTCCGC CCTGAGCAAA  
5281 GACCCCAACG AGAAGCGCGA TCACATGGTC CTGCTGGAGT TCGTGACCGC CGCCGGGATC  
5341 ACTCTCGGCA TGGACGAGCT GTACAAGgga ggtggaGAAG GACGCGGCAG CCTACTGACT  
5401 TGCGGAGATG TCGAAGAGAA CCCTGGCCCT ggtGccaccA TGGTGAGCAA GGGCGAGGAG  
5461 GTCATCAAAG AGTTCATGCG CTTCAAGGTG CGCATGGAGG GCTCCATGAA CGGCCACGAG  
5521 TTCGAGATCG AGGGCGAGGG CGAGGGCCGC CCCTACGAGG GCACCCAGAC CGCCAAGCTG  
5581 AAGGTGACCA AGGGCGGCC CCTGCCCTTC GCCTGGGACA TCCTGTCCCC CCAGTTCATG  
5641 TACGGCTCCA AGGCGTACGT GAAGCACCCC GCCGACATCC CCGATTACAA GAAGCTGTCC  
5701 TTCCCCGAGG GCTTCAAGTG GGAGCGCGTG ATGAACTTCG AGGACGGCGG TCTGGTGACC  
5761 GTGACCCAGG ACTCCTCCCT GCAGGACGGC ACGCTGATCT ACAAGGTGAA GATGCGCGGC  
5821 ACCAACTTCC CCCCCGACGG CCCCATAATG CAGAAGAAGA CCATGGGCTG GGAGGCCTCC  
5881 ACCGAGCGCC TGTACCCCCG CGACGGCGTG CTGAAGGGCG AGATCCACCA GGCCCTGAAG  
5941 CTGAAGGACG GCGGCCACTA CCTGTTGGAG TTCAAGACCA TCTACATGGC CAAGAAGCCC  
6001 GTGCAACTGC CCGGCTACTA CTACGTGGAC ACCAAGCTGG ACATCACCTC CCACAACGAG  
6061 GACTACACCA TCGTGGAACA GTACGAGCGC TCCGAGGGCC GCCACCACCT GTTCCTGGGG  
6121 CATGGACCG GCAGCACCGG CAGCGGCAGC TCCGGCACCG CCTCCTCCGA GGACAACAAC  
6181 ATGGCCGTCA TCAAAGAGTT CATGCGCTTC AAGGTGCGCA TGGAGGGCTC CATGAACGGC  
6241 CACGAGTTCG AGATCGAGGG CGAGGGCGAG GGCCGCCCTT ACGAGGGCAC CCAGACCGCC  
6301 AAGCTGAAGG TGACCAAGGG CGGCCCCCTG CCCTTCGCTT GGGACATCCT GTCCCCCAG  
6361 TTCATGTACG GCTCCAAGGC GTACGTGAAG CACCCCGCCG ACATCCCCGA TTACAAGAAG  
6421 CTGTCCTTCC CCGAGGGCTT CAAGTGGGAG CGCGTGATGA ACTTCGAGGA CGGCGGTCTG

```
6481 GTGACCGTGA CCCAGGACTC CTCCTGCAG GACGGCACGC TGATCTACAA GGTGAAGATG
6541 CGCGGCACCA ACTTCCCCC CGACGGCCCC GTAATGCAGA AGAAGACCAT GGGCTGGGAG
6601 GCCTCCACCG AGCGCCTGTA CCCCCGCGAC GGCCTGCTGA AGGGCGAGAT CCACCAGGCC
6661 CTGAAGCTGA AGGACGGCGG CCACTACCTG GTGGAGTTCA AGACCATCTA CATGGCCAAG
6721 AAGCCCGTGC AACTGCCCGG CTAATACTAC GTGGACACCA AGCTGGACAT CACCTCCCAC
6781 AACGAGGACT ACACCATCGT GGAACAGTAC GAGCGCTCCG AGGGCCGCCA CCACCTGTTC
6841 CTGTACGGCA TGGACGAGCT GTACAAGACT AGTggaggag gaggttctgg tgggtgtTCA
6901 TCCGGAGATT TTGGCAATCC GCTGCGGAAG TTCAAGCTCG TCTTCCTCGG CGAGCAGAGT
6961 GTGGGCAAGA CCTCGCTGAT TACGCGCTTC ATGTACGACA GCTTCGACAA CACGTACCAG
7021 GCGACGATCG GAATTGATTT CCTATCGAAG ACCATGTACC TGGAGGATCG CACTGTGCGC
7081 CTGCAGCTGT GGGATACGGC GGGACAGGAG CGATTCCGCT CGCTGATACC CTCGTACATA
7141 CGCGACTCCA CGGTGGCAGT GGTCGTTTAC GATATCACCA ACACCAACTC GTTCCACCAG
7201 ACCTCCAAGT GGATCGATGA CGTGCGCACG GAGCGGGGTA GCGACGTCAT CATCATGCTG
7261 GTGGGCAACA AGACGGATCT CTCCGACAAG CGTCAGGTGT CCACCGAGGA GGGTGAGCGC
7321 AAGGCGAAGG AGCTTAACGT GATGTTTATC GAGACAAGCG CCAAGGCCGG CTACAATGTG
7381 AAGCAATTGT TCCGACGGGT GGCCGCGGCA CTGCCCGGCA TGGATTCCAC GGAGAACAAG
7441 CCCTCCGAGG ACATGCAGGA GGTTGTGCTA AAGGACTCAC CCAACGAGAC AAAGGATCCC
7501 GAGGGCGGCT GCGCCTGCTA Acgcgtaccg gtCATCATCA CCATCACCAT TGAgtttaaa
7561 cccgctgatc agcctcgact gtgccttcta agatccagac atgataagat acattgatga
7621 gtttggacaa accacaacta gaatgcagtg aaaaaaatgc tttatttgtg aaatttgtga
7681 tgctattgct ttatttghtaa ccatt
```

//